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ON PHYTOPHTHORA PARASITICA NOV. SPEC.

A NEW DISEASE OF THE CASTOR OIL PLANT.

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FOREWORD.

The study of *Phytophthora* on *Ricinus* was undertaken at the suggestion of Dr. E. J. Butler, M.B., F.L.S., the Imperial Mycologist. I have to express my gratitude to him for his ever ready help and invaluable advice which I have received from him. I have also to express my thanks to Mr. F. J. F. Shaw, B.SC., A.R.C.S., F.L.S., the Supernumerary Mycologist, for the microphotographs taken by him.

J. F. D.

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INTRODUCTION.

THE castor oil plant (*Ricinus communis*, L.), a native of India, according to some botanists and according to others a native of Abyssinia and Tropical Africa, is largely cultivated on account of its multifarious uses not only in India, but also in South United States, Southern Africa, Tropical Africa, Algeria, Sicily, Southern France, Brazil, Java, West Indies and other tropical and subtropical regions. In India and elsewhere the castor oil plant is generally grown as a field-border and as a mixed crop but rarely as a sole crop. The plant is extensively cultivated in Surat, Ahmedabad, Dharwar and Belgaum in the Bombay Presidency. In the United Provinces castor is more or less grown everywhere, especially in Allahabad, where it occupies as a sole crop about 1,200 to 1,300 acres, chiefly along the banks of the Jumna. In the Madras Presidency it is widely in cultivation, especially in Salem and Bellary. In Bengal not much castor is to be found. In Assam it is never cultivated as a chief crop but in almost every ryot's garden small areas are allotted to it. The products of the castor oil plant, on account of their varied uses in industry, agriculture and the arts, are of great economic importance. The oil obtained from its seed, the castor oil of commerce, is in great demand. Though a large part of India's produce is locally consumed, still in the year 1910-11 India exported 1,203,007 gallons of the oil, worth Rs. 1,690,455 and 2,148,033 cwts.

of seed worth Rs. 16,499,640. The oil is greatly used in the manufacture of the famous Turkey-red. Again its medical properties secure for it an extensive trade. In many parts of India it is used as a luminant as it gives a bright smokeless light. Especially in Australia and some other places it finds a wide use as a lubricant. It is also employed for dressing tanned hides and skins. The castor oil-cake is an important constitutent of manures. In Assam the plant is cultivated for feeding the *eri*-silk worm on its leaves. The growing cottage *eri*-silk industry will necessitate its wider cultivation for this purpose.

Though the castor oil plant is so widely distributed still no important fungus pest, except the rust,[1] has been recorded as attacking this crop; it has been considered to be comparatively free from fungus enemies, and yet two serious pests occur at Pusa. One is *Phytophthora parasitica nov. spec.*, the other a species of *Cercospora*. The latter is confined only to leaves and so does not prove fatal to the plant. In wet weather this disease is a serious menace to the cultivation of this crop for the *eri*-silk industry, as about ninety per cent. of the leaves of old and young plants are sometimes covered all over with *Cercospora* spots. To a greater or less extent this disease is present all the year round.

In the wet months of August and September *Choanephora* attacks leaves, growing points and tender shoots. This parasite sets up a rot in the affected parts.

There is also an *Alternaria* found occurring on the leaves which might be parasitic.

All these diseases will be dealt with in another paper when their study is completed.

Phytophthora on castor was first found at Pusa in the year 1909 in August. Since then it has been found appearing every year about the end of June, after the rains have set in. Last year (1912) it appeared as early as June on account of the frequent showers of

[1] Ajrekar, S. L. The Castor rust (? *Melampsorella Ricini*, de Toni). Jour. Bombay Nat. Hist. Soc. 1912, p. 1092.

rain we had in April and May. The disease disappears by the beginning of September, at the end of the rains. About nineteen varieties were grown on the Pusa Farm in 1911, but none of them escaped the attack of this fungus, not even the indigenous Patna variety.

In no paper dealing with castor cultivation except in one has any reference been made concerning the high mortality of the seedlings or concerning the characteristic brown leaf spots found on leaves of young and old plants.

Darrah[1] reports that in the year 1889 " a very large number of young castor plants, even those from the indigenous Cachar seed, had damped off " on account of damp and excessive rain. Mr. Mackenzie, who was in charge of the *eri*-silk experiments, observed that the seedlings damped off and died " before they matured a sufficiently hard skin or bark on their stems."

This damping off of seedlings in water-logged places is what I have found on the Pusa Farm to be due to *Phytophthora parasitica*. In all probability therefore *Phytophthora* was the cause of the large destruction of castor seedlings of Mr. Mackenzie's crop in 1889.

This parasite is the most injurious of the fungal parasites of castor; it destroys seedlings and attacks leaves of older plants. My thanks are due to Mr. M. N. De, the Sericulture Assistant at Pusa, for carrying out experiments to see the effect of feeding *eri*-silk worms on leaves attacked by *Phytophthora*. He informs me that the worms refused to eat the diseased portions of the leaves, and that their growth when fed on diseased leaves was very slow as compared with those fed on healthy leaves.

In the case of a monsoon (Kharif) crop the most critical period in its life history is when the seedlings are about six to eight inches high and when their stems are quite tender. In low-lying water-logged and badly drained fields about thirty to forty per cent. of the seedlings damp off. The first indication of the disease is the appearance of a roundish patch of an unhealthy dull green colour on both the surfaces of a cotyledon. It soon helplessly

[1] Darrah, H. Z. The *Eri*-silk of Assam, 1890, p. 15.

hangs down from its point of attachment. The disease spreads from the leaf to the petiole, thence to the stem and the growing point, killing the seedling. In older plants the disease in the field is localised on the lamina or leaf-blade. As the diseased spot gets older it turns yellowish and then brown. It spreads concentrically, and in old spots forms concentric brown rings enclosing lighter brown areas (Plate I). They are surrounded on the upper surface by a yellowish green or dull pale green ill-defined border. On the under surface the old spots have a brownish grey and ill-defined border which is relieved occasionally with very minute white specks. The brown central portion of the spots is always very sharply contoured. The diseased areas either remain isolated or become confluent, often covering almost the whole of the leaf. In case of confluent spots the successive periods of their growth are not marked by concentric rings but by wavy margins of different shades of brown. The spots are not limited by the main ribs, even in the case of big mature leaves. The diseased leaves have a tendency to fall off prematurely. Growing points and leaf-buds of plants of all ages are protected from the external invasion of the disease by the waxy sheathing stipules which completely cover them. Petioles of big leaves, stems with thick epidermis, flowers and fruits, have been found till now in nature to be immune from the ravages of the fungus.

On the under surface of the leaf is seen with the aid of a pocket lens a sparse white web of threads originating from the unhealthy green coloured portion of the diseased spot. These are the long and unbranched sporangiophores, measuring from 35 to over 500μ, as a rule 100 to 300μ. They emerge from within the leaf as a rule through stomata or between the cell walls of two contiguous cells (Plate II, Fig. 1). They have been occasionally found to make their way out by breaking through the upper wall of the epidermis. They make their exit from the leaf singly, rarely in twos or threes but never in clusters.

In the petiole and stems it is a purely physical matter for the hypha to make its way out of the tissues. Just underneath the upper wall of the epidermis the hypha swells into a globular head and

under its pressure the wall gives way (Plate II, Fig. 6). In petioles and in stems the hypha emerges singly as a rule, as in the case of leaves, but occasionally a cluster of four or five have been found to come out from the same point (Plate II, Fig. 7).

The mycelium within the tissues is both intracellular and intercellular. Wherever an intracellular hypha passes through a wall there is invariably a constriction of the hypha (Plate II, Fig. 2). Haustoria are very scarce. Hartig[1] has described them to be button-shaped in *Ph. Fagi* (*Ph. omnivora* de Bary), Coleman[2] finger-shaped in *Ph. omnivora*, var. *Arecæ*, Klebahn[3] short thick and finger-like in *Ph. Syringæ* and *Ph. omnivora*, while de Bary says, " Bestimmte Haustorien sind nicht vorhanden " but in *Ph. parasitica* both finger-shaped and button-shaped haustoria have been found (Plate III, Figs. 2 B and 3) ; they branch very rarely. Whether these bodies which we call haustoria are true haustoria or the initial stage of branches of the intracellular hyphæ arising from the intercellular mycelium remains an open question. No cellulose cap has been observed at the tips of these finger-like or button-shaped bodies which would leave no doubt as to their identity. In Plate III, Fig. 2, A is distinctly a branch of the intercellular hypha but B may be a haustorium or a branch like A in a very early stage ; but, on the other hand, in Plate III, Fig. 4, A appears to be a branched haustorium.

When a diseased petiole or stem is cut transversely, the ring of fibro-vascular bundles becomes prominent by its black or brown colour on account of the fungus choking the vessels (Plate II, Fig. 2). *Ph. omnivora* de Bary and *Ph. omnivora*, var. *Arecæ* Coleman have been known to attack the vascular bundles. The hyphæ of *Ph. parasitica* enter and leave the vessels of the fibrovascular bundles through their unthickened portions. The hypha at the point of entrance or exit generally swells, the thin

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[1] Hartig, R. Untersuchungen aus dem forstbotanischen Institut zu München, 1880, p. 41.

[2] Coleman, L. C. Diseases of the Areca Palm. Dept. of Agric., Mysore State, Myc. Ser. Bull. No. 2, 1910, p. 82.

[3] Klebahn, H. Krankheiten des Flieders, 1909, pp. 42 and 43.

membranous portion gives way, possibly under pressure and the fungus continues its course inwards or outwards as the case may be (Plate II, Fig. 3).

Internal sporangia have often been observed in the tissues of leaves, stems and fruits. They have not been found either to germinate conidially or to produce zoospores in leaves and stems, but in a few cases they have been observed to germinate conidially in the soft tissues of the pericarp of the fruit (Plate V, Fig. 15). Of what use they are to the parasite it is difficult to say. They are known to be formed in the leaf within twenty-four hours after inoculation. Similar bodies have been found in the leaves of potatoes attacked with *Ph. infestans*[1].

I have failed up to now to find typical oospores in nature. It is possible that they are formed, as in some artificial media they are readily produced, but they may be very few and so escape detection. Very rarely on mashing old leaves boiled in caustic potash resting bodies have been found which might be parthogenetic oospores.

Infection Experiments on the Castor Oil Plant.

From the many series of inoculation experiments carried out in the laboratory it has been found that leaves young and old most readily fall victims to the attack of the parasite. Before it was taken in pure culture inoculations were made in either of the two following ways:—(1) A bit from a diseased portion of a leaf from the field was placed with a drop of water on either of the surfaces of a leaf of a healthy plant which was then covered by a bell jar to keep it in a moist atmosphere. After about six hours the diseased piece used for inoculation was removed. (2) A diseased leaf from the field was kept for twenty-four hours slightly immersed in water in a covered Petri dish. The fungus grew out on the surface of the water in the Petri dish and produced sporangia in abundance; from the surface not in contact with water an aerial growth was produced. Either zoospores suspended in water or a little of the mycelium

[1] Jones, L. R., Giddings, N. J., and Lutman, B. F. Investigations of the Potato Fungus *Phytophthora infestans*. U.S. Dept. Agric., Bureau of Plant Industry, Bull. No. 245, 1912, p. 28.

were used for inoculating healthy leaves. When the fungus was taken in culture, zoospores suspended either in distilled water or in sterilised tap water were invariably used for the various inoculations. Whatever was the technique employed for the inoculation the result was always the same. The effect of the inoculation was clearly perceptible within twenty-four hours by the inoculated area taking a dull unhealthy green colour. Sporangia were produced in about forty-eight hours after inoculation, when the inoculated leaf was kept moist by occasionally spraying it with water. The inoculated leaves could be easily induced to produce sporangia by suspending them in tap water. They readily discharged their zoospores when transferred to a drop of fresh water. These zoospores were used to inoculate a new set of leaves of healthy plants. The inoculation took as readily as in the previous experiments. In the case of leaves of tender seedlings and of very young leaves of bigger plants the disease spread from the lamina to the petiole, thence to the stem.

From these infection experiments it is found that the germ-tubes of the zoospores penetrate the leaf three to five hours after they are sown on it. The penetration takes place on either of the surfaces and is not accomplished necessarily through a stoma. They may even break through the upper wall of the epidermal cells or penetrate between the cell walls of two neighbouring cells (Plate III, Figs. 5-11). What factor stimulates the germ-tube to enter the host plant in one way or the other is difficult to say ; very often it has been found to cross over a stoma without entering it (Plate III, Fig. 10). Before the germ-tube enters the leaf its end is slightly enlarged as a rule.

We have seen that in nature the fungus is confined to seedlings and to leaves of older plants. Stems of tender seedlings about six to eight inches high are found diseased in nature. When a stem of a healthy tender seedling is inoculated by means of zoospores suspended in a drop of water, the inoculated area soon turns brown. The disease extends in both directions, ultimately reaches the

growing point and thereby kills the seedling. If the seedling is kept in a very moist atmosphere the inoculated stem produces in forty-eight hours a white fluffy aerial mycelial growth, bearing sporangia. The hyphæ project about a millimetre or two from the stem. But stems of plants out of the seedling stage seem to be immune from the attack and artificial inoculations have proved unsuccessful on these. When the ordinary method of inoculating, by means of putting a drop of water containing freshly discharged zoospores on these stems failed, Klebahn's[1] method, employed by him in inoculating leaf buds of lilac plants, was tried. The stems of young plants about three to six months old were surrounded by a jacket of distilled water, containing motile zoospores, in a glass tube about four inches long and three-fourths of an inch broad, the lower end of which was plugged by a rubber cork cut into two equal halves and having a central bore just big enough to enable the two halves to surround the stem without injuring it. This end was made water-tight by means of moulding wax (Plasticine) or paraffin ; the upper end was kept open. A young seedling was inoculated in the same way. The result was that the stem and the base of the petioles of the seedling in two days distinctly showed marks of successful inoculation and in four days it wilted. The photo (Plate X), taken on the fourth day after inoculation, shows the progress of the attack from the petiole to the leaves. The plants three to six months old remained quite healthy. Only one plant out of half a dozen such inoculations on plants with matured bark, showed a small blackish patch about a couple of centimetres in length, in two days after inoculation. As the diseased area was found not to grow further, even after a week, sections were made at this place. A few hyphæ were found confined to the epidermal cells only. They were unable to grow further into the stem and the consequent lack of food killed them. The inoculation failed even when the stem was wounded. The reason of the parasite not attacking stems of plants as soon as they are out of the seedling stage is

[1] Klebahn, H. *loc. cit.*, p. 55.

not far to seek. The tender stem of a seedling has an uncuticularised epidermis, the outer wall of which readily permits the hyphæ to penetrate it, the cortex is soft and juicy and therefore easily succumbs to the ravages of the parasite; its growth is not entirely checked on reaching the vascular bundles which, as we have seen, are attacked through the unthickened portions of their vessels. On the other hand, the stem of an older plant presents obstacles in its way from the very beginning. The outer wall of the epidermis is highly cuticularised and so wards off an attack of the hyphæ and even if they get into the stem through stomata, the germinating tube cannot go further on account of the thick-walled and closely packed collenchymatous cells of the cortex. Apart from these reasons of physico-chemical importance there may be very possibly others of physiological importance. Colour is lent to this by the disease confining itself to the lamina of a mature leaf of young plants and not spreading to the petiole and thence to the stem. I have succeeded up to now in inoculating petioles of only very young leaves. The inoculation was made by placing distilled water containing motile zoospores in a cup of moulding wax (Plasticine) which surrounded a part of the petiole at the node and parts of the internodes above and below it. The stem did not take the inoculation but the petiole did, in two days. The stem was then attacked through the latter. The hyphæ which entered from the petiole extended up the stem and killed the growing point. The growth of fungus in the opposite direction was very slow. In these various experiments it was found that not only under field conditions is the leaf bud safe from the attack of the parasite, but also when inoculated aritificially, on account of the sheathing stipules.

Infection experiments have also been carried out on flowers and fruits. For these experiments as well the inoculation material was zoospores from pure cultures, suspended in either distilled or tap water. Stalks bearing flowers and fruits were stood in water in flower vases; drops of water containing motile zoospores were placed on the flowers and fruits. After the inoculation the flower vases were kept in moist chambers. Stalks of flower and fruits kept as "checks"

were placed in exactly similar conditions. Male flowers took the inoculation in a day, turning brown and then black. The branched stamens were cobwebbed by hyphæ, rich in sporangia. Green fruits in two days turned black and became soft at the point of inoculation. The disease then spread over the whole fruit and caused it to rot. Sporangia and "resting" conidia were abundantly produced. The attack was not confined only to the outer soft portion of the pericarp, but the hard woody portion also was found to contain stray strands of hyphæ which made their way through the lignified cells by means of a very fine projection which bored through the hard cell wall (Plate II, Fig. 4). A fine web of mycelium was found in the cocci between the seed and the endocarp. The fungus extended there through the lignified cells or, as often happened, through the dissepiments of the loculi. The fleshy outgrowth on the seed, the caruncle, was then attacked by the hyphæ and through it the immature seed. The hyphæ completely filled the caruncle. They invariably traversed the cells through the pits in their thick cellulose walls. The course of the hyphæ in the seed coat was difficult to trace. Inoculations on dried fruits invariably failed.

Though in the laboratory fruits and flowers are so virulently attacked by the parasite, still in nature they have been found to be entirely immune from its attack. The cause for this is solely to be attributed to the climatic conditions prevalent at the time of fruiting. The rainy (Kharif) crop fruits in October, November and December, a period when the fungus has completely disappeared from the fields. The winter (Rabi) crop fruits in April and May, when the temperature is too high for the fungus to grow.

The following experiments were undertaken to see how far soil inoculations prove successful.

On account of the frequent showers of rain in April and May last year (1912) the disease broke out early in June in self-sown plants in the castor plots of the previous year. About fifty to sixty per cent. of the seedlings were diseased. All the seedlings were removed from the field early in July. On the 17th three pots (Lot A) were filled

with earth from this diseased area. Care was taken to have the topmost layer of the earth in the field uppermost in the pots as well. Three seeds which were just germinating were removed to three pots with a big lump of earth (Lot B). Ten seeds were sown in each of the three pots of Lot A. On the 21st they germinated. On the 22nd one seedling of Lot B was attacked with *Phytophthora*. On the 23rd morning one of the seedlings of Lot A was suddenly found to have given way at the collar, at which place there was found an elliptical pale grey patch surrounded by a black brown border which in turn was enclosed by a light brown and not sharply defined ring. Sporangiophores were produced in a large quantity, breaking out through the epidermis at any point. On making sections internal sporangia were also found as far in as the pith. The cells of the pith and cortex were traversed by palmately branched or fasciculated hyphæ, the like of which have till now been observed only in some of the artificial media; wherever these hyphæ were found, the cell walls between two contiguous cells were partly or wholly dissolved (Plate II, Fig. 5). These palmately branched hyphæ have also been observed by Mangin[1] in *Plasmopora viticola*, *Pl. ribi*, *Pl. nivea* and some other *Peronosporaceæ* and according to him by MM. Milladet, Prillieux and Cavara as well, but these hyphæ, Mangin has found only in tissues surrounded by fibro-vascular bundles. He says, "cependant les tissus compacts qui entourent les nervures et qui cantonnent le mycélium dans des régions limitées, ne constituent pas une barrière infranchissable. En ces points, le mycélium s'aplatit et forme des lames ou des palmettes très élégamment ramifiées, qui s'insinuent dans les méats les plus étroits et parviennent même, soit mécaniquement, soit en digérant la substance intercellulaire, à dissocier un peu les cellules qui leur opposent de la résistance. La moindre fissure, la moindre fente, suffisent pour que le mycélium franchisse l'obstacle et pénètre dans une région saine; là, il évolue rapidement en pleine liberté au moyen des éléments nutritifs qu' il

[1] Mangin, L. Recherches sur les Péronosporées. Bull. Soc. d'Hist. Nat. d'Autun, VIII, 1895, p. 72.

trouve en abondance." I have found these palmately branched hyphæ in the cortical parenchymatous cells and growing away from the fibro-vascular bundles. The leaves and the growing point were not attacked when the seedlings gave way at the collar on being diseased. On the 24th another seedling from Lot B was diseased. The third remained healthy for a week more and then this part of the experiment ended. The seedlings from Lot A got attacked at the surface level of the soil one by one, till the majority of them died. The attack was never found to come from the roots upwards. It invariably began at the surface level of the earth in the pots.

All the pots of the Lots A and B, with some of the diseased seedlings were then kept in a moist condition in the laboratory. On the 30th of August one pot from Lot A was again sown with ten castor seeds. By the 5th of September eight seedlings died of the attack on the surface level. One of the two remaining seedlings was slightly attacked at the same place, but it succeeded in the struggle for existence. The other was not at all attacked.

It is needless to say that for all these experiments "checks" were kept in exactly the same condition, except that the earth was not from diseased areas.

On the 30th of September a second pot of Lot A was sown with castor seeds, *i.e.*, two months after the seedlings in this pot died of the attack. The seeds germinated after a week, and the seedlings grew into big plants without any being in the least diseased. This shows that the fungus in the soil was not able to retain its vitality for two months.

hese experiments have proved interesting first because seedlings have been found to be attacked at the surface level of the earth got from diseased areas, while in the fields the attack has not been found to be at the surface level; secondly, because of the presence in the diseased tissues of the seedlings of palmately branched hyphæ, such as have been found growing in some culture media, while in diseased leaves and stems obtained from

the fields or by inoculations in the laboratory such hyphæ have never been seen.

Pure Culture.

Klebahn[1] succeeded in getting a pure culture of *Ph. omnivora* by inoculating agar in a Petri dish, directly from an infected beech seedling, but the *Phytophthora* on *Ricinus* could not be taken into pure culture by this method or by the ordinary poured plate method successfully employed by Rorer[2] for getting a pure culture of Cacao *Phytophthora*, possibly because this fungus gives only a submerged growth on glucose meat-extract agar, as was found when working with pure cultures. *Ph. infestans*[3], *Phaseoli*[4] *omnivora*, var. *Arecæ*[5] and *Colocasiæ*[6] have been taken into culture by cutting aseptic slabs from the diseased tubers, fruits and rhizomes ; a pure culture of *Ph. Syringæ* was got by Klebahn[7] by incubating the rind of a diseased stem of Syringa ; evidently these methods could not be employed in the present case as in nature the fungus is found only on stems of tender seedlings six to eight inches high and on leaves. Flaming killed both the host and the parasite. Diseased leaves were suspended over sterilised pieces of carrot, potato, and in moist chambers on agar plates so as to allow conidia to drop on to the slabs and agar plates ; this method has been used with success by Rolfs[8] in getting pure cultures of *Rhizoctonia* from the basidiospores of its *Corticium* stage ; with *Ph. parasitica* the media always got contaminated with bacteria and other saprophytes. Raciborski[9] says he has grown *Ph. Nicotiana* in artificial culture but does not describe his technique.

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1 Klebahn, H. *loc. cit.* p. 70.

2 Rorer, J. B. Pod-Rot, Canker and Chupon-Wilt of Cacao caused by *Phytophthora* Sp., Bull, of Trinidad Dept. of Agr., IX, No. 65, 1910, p. 14.

3 Clinton, G. P. Downy mildew or blight (*P. infestans* (Mont) de Bary), of Potato, II, 29th Report of the Connecticut Exp. Sta. 1905, p. 278.

4 *Ibid.*, Downy mildew or blight (*P. Phaseoli* Thaxt.) of Lima beans, ib., p. 278.

5 Coleman, L. C. *loc. cit.*, p. 69.

6 At Pusa. An account of this species by Dr. E. J. Butler and Mr. G. S. Kulkarni is in the press.

7 Klebahn, H. *loc. cit.*, p. 37.

8 Rolfs, F. M. Potato Failures, Bull. 91, Colorado Expt. Sta. 1904, p. 10.

9 Raciborski, M. Parasit. Alg. Pilze Javas, Theil III, 1900, p. 5.

Trow[1], when working with *Pythium ultimum*, found it only necessary, in order to get a pure and copious growth of the fungus, to sterilise potatoes in suitable glass pots and inoculate them with a trace of the mycelium from the aerial growth developed on inoculated cabbage and rhubarb leaves projecting above the surface of water in Petri dishes. It, therefore, at first, seemed easy to get similarly a pure culture of *Ph. parasitica* from the aerial mycelial growth obtained about half a centimetre high in 24 to 48 hours by keeping a freshly diseased leaf in a Petri dish containing a little tap water, but when inoculations were made on sterilised potatoes, carrots, bread paste, acidified bread paste, glucose meat-extract agar, acidified glucose meat-extract agar, prune juice agar and *Ricinus* leaf juice agar, it was invariably found that bacteria contaminated the media and overgrew the fungus. Attempts were made to get a bit of a hypha free from bacteria by nipping it off just below its apex by means of a hot platinum needle but the hypha being unseptate the bit on the needle lost its protoplasm from its cut end; thus while getting rid of bacteria its vitality was destroyed. A living potato washed with formalin and cut into two by a sterile knife was inoculated on its cut surface. An aerial mycelial growth soon developed but again was not free from bacteria.

A pure culture was ultimately got by means of the following technique.

A diseased leaf was got from the field. It was well washed with sterilised water to get rid of foreign matter as much as possible and kept in a covered sterilised Petri dish containing a little sterilised tap water. The *Phytophthora* hyphæ spread out on the surface of the water and bore a crop of sporangia the next day. They readily discharged their zoospores in a drop of sterilised tap water. This drop was used to inoculate a clean well washed newly opened leaf of a seedling grown in the laboratory, thereby further minimising the presence of saprophytic fungi. The seedling was kept in a moist

[1] Trow, A. H. Observations on the Biology and Cytology of *Pythium ultimum*, n. sp., Ann. Bot., XV, 1901, p. 279.

atmosphere by covering it with a bell jar. The next day the inoculated leaf was plucked and immersed in 2·5 per cent. formalin for half an hour, it was then well washed with sterilised water and transferred to a sterilised covered Petri dish containing a little sterilised tap water. From the inoculated spot an aerial mycelial growth of *Phytophthora*, free from other fungi but not from bacteria, developed in 24 to 48 hours. It was found essential to keep the leaf in contact with water to get the aerial growth. A portion of the aerial mycelium was then transferred by means of a sterilised platinum needle to a piece of pith soaked in *Ricinus* leaf juice prepared as follows : About 10 to 15 grammes of green castor leaves were boiled for an hour in 100 c.c. of distilled water. One or two pieces of pith in an Erlenmeyer flask were soaked in some of the filtered decoction, enough to leave very little of it at the bottom of the flask after sterilisation. After two or three days a sparse woolly growth projected about a centimetre high from the upper surface of the pith. These hyphæ were free from bacteria and bore no sporangia except occasionally in old cultures, but when a bit was transferred to water, it bore innumerable sporangia the next day. If the flask contained much liquid the pith was soon overgrown with bacteria and the growth of the fungus retarded. It was now only necessary to inoculate a suitable culture medium with a bit of the mycelium growing on the pith. Such a medium was found in French bean (*Phaseolus vulgaris*) juice agar which gave in two days a pure, rich, healthy culture.

Morphology of the Fungus in Culture.

The comparatively young mycelium consists of thin, not much branched, unseptate hyphæ. The protoplasm within them is finely granular ; but as they grow older they become very broad, up to 9μ ; the granules become coarser and aggregate into groups or irregular masses, the contents lose their homogeneity ; at a still later stage septa are formed, but they are far between. In very old cultures they are formed fairly frequently. In these cultures branches have been found to be rather uniformly cut off from their mother

hyphæ at the point of origin (Plate V, Fig. 4e). They are generally curved, either concave or convex ; they are more or less thickened either on one or both sides by the deposition of cellulose on them. The thickening is either elliptical or conical (Plate V, Fig. 4). It resembles callose caps on sieve plates. The cellulose thickening when stained with Schulze's solution does not take a homogenous blue or violet colour but different shades of blue or violet. This shows that the cellulose on the septa has been deposited in layers. On rare occasions branches have been found to grow through septa (Plate V, Fig. 6). Cellulose ingrowths have been found to form false septa in hyphæ (Plate V, Fig. 5). They differ from the ordinary septa in not having their characteristic curve, in being broadened at the ends and not in the middle, and in not being laid down in plates, but in being ingrowths from the inner layer of the hypha walls. The false septa are formed either by two cellulose pegs growing in from the opposite walls and meeting at some point (Plate V, Figs. 4d and 5c) or by cap-like depositions of cellulose on the ingrowths (Plate V, Fig. 5b).

The sporangia are borne on simple stalks, but in water cultures and in certain media the stalks are as irregularly branched as those of *Ph. omnivora* and of *Ph. omnivora*, var. *Arecæ*. The sporangia are generally apical as in a typical *Phytophthora ;* but they are often intercalar and sometimes lateral as well ; the end of a hypha swells as if it was forming a sporangium, but for some unknown cause the swelling does not increase and produces three or four short lateral branches, each of which terminates in a sporangium. Occasionally an apical branch also arises from the swelling, which prolongs the vegetative growth of the original hypha. The sporangia are generally pear-shaped but intercalar sporangia are usually round (Plate V, Fig. 1b) ; they do not vary much in shape but they vary a great deal in size. The variation is between 11-60 × 10-45μ, on an average 25-50 × 20-40μ, and consequently the number of zoospores in them is very irregular. They have from 5 to 45 zoospores, on an average 30. Sporangia and zoospores from diseased leaves are found to be smaller than those from pure cultures. Sporangia have usually one papilla

vertically opposite the stalk, but some sporangia have been found with two (Plate IV, Fig. 36). In such cases the discharge of zoospores does not necessarily take place through the apical papilla.

Sporangia fall off from their sporangiophores with or without any vestige of their stalks. Sporangia from a healthy culture, when suspended in water and in ordinary day-light, discharge their zoospores within five minutes. Sporangia from cultures made in May and June 1912, when the temperature was very high, did not discharge their zoospores so readily. They required to be suspended in water for half an hour to make them discharge their zoospores, but when they were suspended in water and kept in an incubator at 25° C., zoospores were emitted in five minutes. The same result was obtained by adding a small piece of ice to water containing sporangia. Cultures made in these hot months were occasionally daily put in an incubator at 25° C. They produced sporangia luxuriantly, which discharged their zoospores in ordinary tap water or distilled water in five minutes at room temperature. Submerged sporangia from French-bean juice agar and oat juice agar discharge their zoospores even when embedded in the media. These zoospores, after leaving the sporangium crawl about for a few seconds in the film of water expressed from the agar before rounding themselves off, and germinate like other zoospores. Light seems to influence the formation and emission of zoospores. A dozen healthy hanging drop cultures were kept in a dark cupboard and a dozen similar cultures left on the working bench in ordinary day-light. Within half an hour all the mature sporangia in the cultures left on the bench had discharged their zoospores while there was no change in those kept in the dark; but the next day sporangia in these cultures had germinated conidially, giving a highly branched mycelium (Plate III, Fig. 1). Both these sets of cultures were kept under observation for three days. The cultures kept in the dark formed no new sporangia but in those kept on the working bench the sporangia that had not discharged their zoospores had germinated conidially and bore secondary sporangia (Plate IV, Fig. 37); and the new plants got by germination of zoospores had also formed sporangia.

Varying temperature, alternate light and darkness and moisture are essential factors in the formation of sporangia. Cultures kept for months in a dark cupboard at varying room temperature and in incubators at the constant temperatures of 20° C. in summer and 35° C. in winter, produced luxuriant mycelium but remained sterile. But when they were removed to an ordinary room in ordinary atmospheric conditions, sporangia were soon formed. Inoculated castor seedlings kept in a dry place in the laboratory produced no sporangia but those kept in the laboratory compound on misty nights and those kept in the laboratory but occasionally sprayed with water, gave sporangia in forty-eight hours.

An immature sporangium has a single vacuole about 10-12μ in diameter. It glides from place to place in the sporangium by very slow, almost imperceptible "amœboid" movements. As it contracts and expands the size of the sporangium has been found to be decreasing and increasing. The formation of zoospores is made faintly evident by the segmentation of the protoplasm into so many units while the vacuole is still present. At a later stage the vacuole disappears and the zoospore origins become more prominent. At this stage they have no vacuoles. They then become more coarsely granular. The sporangium looks rather dark. A little later in each of the zoospores a vacuole is seen appearing and disappearing. The papilla is now very highly refractive. The contents of the sporangium have contracted a little from the inner wall. A slight pulsating movement in it heralds the approaching discharge of the zoospores. There is a slight spasmodic rotation of the whole mass. Individual zoospores are restless, they contract and swell. There is also a slight motion visible in their granular protoplasm. The inner wall opposite the papilla is occasionally pushed into it by a zoospore striking against it. The discharge of the zoospores takes place in either of the following two ways :—

1. The papilla dissolves or bursts under pressure. The zoospores are immediately discharged but sometimes a little granular fluid flows out before them. Sometimes the papilla dissolves before

the zoospores move about in the sporangium or make any effort to escape[1] (Plate IV, Fig. 4).

2. The papilla swells or is blown into a hemisphere by the inrush of the zoospores (Plate IV, Figs. 1 and 2). It disappears before all the zoospores have left the sporangium (Plate IV, Fig. 3). Those that get into it remain stationary, huddled together for a few seconds, before dashing away. The swollen papilla can be distinctly seen by treating a discharging sporangium with iodine. This mode of discharge has been observed in *Pythium palmivorum*.[2]

The zoospore escapes from the sporangium by contracting its anterior end and squeezing it through the opening; it then swells up, the zoospore becoming dumb-bell shaped. Then the posterior end contracts and passes to the outside. Its vacuole either disappears and re-appears as soon as the anterior end of the zoospore is out of the sporangium, or divides into two, one half going to the front portion and the other to the hinder and both re-uniting when the zoospore is completely out of the sporangium, or along with the contraction of the zoospore the vacuole also contracts, looking like an hour-glass, when the zoospore is partly out of the sporangium. Each zoospore as it makes its escape from the sporangium generally drags out the one behind it. It is not always that the zoospores succeed in getting out of the sporangium. Those that do not find their way out, round themselves off and may germinate *in situ*.[3] Zoospores in a sporangium which for any reason get separated from those that are escaping from it, find it difficult, and frequently altogether fail, to get out.

The zoospores, as a rule, escape as distinctly defined units but occasionally in clumps of several united together, moving about by amœboid movements before getting separated from each other.

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[1] *C.f.* Hartig, R. *loc. cit.*, p. 44.

[2] *C.f.* Butler, E. J. Bud-rot of Palms in India. Mem. Dept. Agric. India, Bot. Ser. III, No. 4, 1910, p. 254.

[3] *C.f.* Hartig, R. *loc. cit.* Coleman, L. C., *loc. cit.*, p. 62.

In rare cases two zoospores have been found attached together by a protoplasmic neck (Plate IV, Fig. 6a). The whole mass has been observed to have only two cilia, one at each end. The two zoospores make an effort to get free from each other. They forcibly pull at each other and violently twist round and round the neck, which elongates and consequently becomes thinner. It finally snaps, the zoospores immediately dash away, with a cilium at one end and a protoplasmic thread at the other. This is probably not a formation of two uniciliate zoospores by the fission of one bi-ciliate swarm spore, as found by Atkinson in *Artotrogus* (*Pythium*) *intermedius* (de Bary)[1]; but merely an incomplete breaking up of the original protoplasm in two units of zoospores. In Atkinson's case the movement of the bi-ciliate zoospore becomes slower five or ten minutes after discharge and "finally it nearly ceases and the body undergoes plastic movements resembling somewhat that of an amœba. At first this amœboid movement is irregular, but after a few minutes it assumes a definite character which tends to cut the organism into two." His figures show that the bi-ciliate zoospores have a single vacuole.[2] What I have found is the appearance of two distinct units connected by a narrow neck at the time of discharge, each unit possessing a vacuole (Plate IV, Fig. 6a); the whole mass being almost twice the size of a normal zoospore; its movement is at no stage "amœboid," and the two units get separated by the snapping off of the protoplasmic neck, the result of the hard pull that they have at each other. I have found two units thus united together hardly half a dozen times while Atkinson has found the breaking up of bi-ciliate zoospores into two uniciliate ones taking place as a rule. In one case, along with normal zoospores, a motile mass of protoplasm was discharged from a sporangium at 10-15 A.M. (Plate IV, Fig. 26). Its shape was that of a roughly drawn pentagon with a distinct cilium at each of its angles. It whirled round and round a single point as if one of its cilia, which was not visible, had

[1] Atkinson, Geo. F. Damping off, Cornell Univ. Agric. Expt. Stat. Bull. 94, Bot. Division, 1895, p. 249.

[2] *Ibid.*, pl. II, fig. 24, p. 247.

got entangled with some foreign body. It evidently tried to set itself free. At 10-20 four vacuoles appeared, at 10-25 it succeeded in disentangling itself and suddenly darted off. At 10-30 its motility ceased, it lost its cilia and rounded itself off. At this stage only one vacuole was visible. It measured $15 \times 12\mu$. Its endospore and exospore were quite distinctly visible (Plate IV, Fig. 27). The former was hyaline and refractive, the latter dark. At 10-45 it began to germinate. It produced three germ-tubes. Plate IV, Figs. 27 to 34 show its progress till 3 P.M., after which there was no further development, possibly on account of lack of food. This motile mass of protoplasm, having five visible cilia and going through the ordinary life cycle of a normal zoospore and germinating by giving out three germ-tubes, possibly consisted of three undifferentiated zoospore constituents. Dr. Butler[1] has observed in *Pythium palmivorum* two or more spores remaining united after leaving the sporangium and germinating normally after coming to rest, but they have only a single wall.

The zoospores are of the ordinary *Phytophthora* type and measure $8\text{-}12 \times 5\text{-}8\mu$ in their motile state, when they come to rest they measure $7\text{-}11\mu$. They are bean-shaped with a pointed anterior; arising from the hilum are two unequal cilia, the anterior one being distinctly shorter, as found in *Ph. Syringæ*[2] and *Ph. omnivora*, var. *Arecæ*[3] (Plate IV, Fig. 6). After swimming about for twenty minutes to two hours they come to rest. Their movements are very brisk for the first fifteen minutes; they move about even after assuming the spherical shape, the movement then being not very energetic. The rounded zoospore after rolling about for a few seconds comes to a dead stop to all appearances, but quite suddenly after a few seconds it dashes away, presumably in search of another suitable resting place. This fitful and sudden movement goes on for about five minutes more and then the spore comes to a dead halt. In fifteen minutes germination takes place

[1] Butler, E. J. Bud-rot of Palms in India. Mem. Dept. Agric. India, Bot. Ser. III, No. 4, 1910, p. 255.

[2] Klebahn, H. *loc. cit.*, p. 51.

[3] Coleman, L. C. *loc. cit.*, p. 63.

generally by one and rarely by two germ-tubes (Plate IV, Figs. 8 to 25). The germination does not seem to be affected by light or darkness. Two sets of experiments were carried out to see the action of light on the germination of zoospores. A little bit of mycelium from a pure culture was transferred to tap water. Within ten minutes an army of zoospores was actively moving about. They were transferred to twelve slides, half of which were kept in a moist chamber exposed to ordinary day-light at laboratory temperature, while the rest were kept in a moist chamber in a dark cupboard at the same temperature. In two hours all the spores had germinated equally well in both the sets.

The germination of the sporangia takes place in three different ways. (1) When they are placed in a drop of fresh water their protoplasmic contents break up into the constituent zoospores, which after escaping from the sporangia germinate readily within half an hour. (2) They may give rise to one or more short germ-tubes forming secondary sporangia, which may either form zoospores or tertiary sporangia at the end of short germ-tubes. It is not unusual to find three or four generations of sporangia linked together by short germ-tubes (Plate V, Fig 1). This mode of germination is generally found in old cultures and in those not kept in pure water. (3) They germinate conidially giving rise to a mycelium.

In cultures and on inoculated castor fruits a second spore form has been found, the " resting " conidium or so-called " chlamydospore." This " resting " form of conidium has been observed in the Cacao *Phytophthora* by Rorer[1] and apparently also by Petch[2], and in *Pythium palmivorum*[3] by Dr. Butler. The " resting " conidia are usually spherical, thick and smooth walled, yellow in colour, terminal, lateral or intercalar, 20-60μ in diameter and contain a lot of oil globules (Plate V, Figs 1a and 7 to 13). Intercalar " resting " conidia are formed in two ways. (1) In the course of a hypha, swelling takes

[1] Rorer, J. B. *loc. cit.*, p. 13.

[2] Petch, T. Cacao and Hevea Canker, Cir. and Agric. Journ. Royal Bot. Gard., Ceylon, V, No. 13, 1910, p. 153.

[3] Butler, E. J. *loc. cit.*, p. 255.

place in both its walls or in one at first, forming a round or elliptical enlargement which is then cut off by two septa (Plate V, Figs. 11 and 13). The thickening of the wall begins at a very early stage. (2) A terminal swelling appears on a young hypha, which soon becomes cut off by a septum from the swelling ; the growth of the hypha is continued either in a prolongation of its original course or in a new direction by the swelling putting out a new hypha. This is not a germination of the swelling by a germ-tube, but it is merely a vegetative growth as the swelling is immature and develops into a fully mature thick-walled yellow " resting " conidium after the new hypha has grown out. It is capable of germinating independently. In cultures " resting " conidia are borne both on the aerial and submerged mycelium. They are formed both in healthy and unhealthy cultures. Extremes of temperature, which retard the formation of sporangia, do not retard the formation of " resting " conidia. Cultures made in May and June last (1912) produced a poor crop of sporangia on account of the excessive heat in these months but were rich in " resting " conidia. Very old cultures, about nine months old, in which all sporangia and hyphæ were dead, contained living " resting " conidia. The " resting " conidia do not necessarily require a period of rest before germinating. They germinate by one or more germ-tubes quite readily (Plate V, Fig. 8). When " resting," conidia are sown in water, germination takes place within twenty-four hours. Very frequently the germ-tubes are swollen at the base. The germ-tubes either directly produce sporangia (Plate V, Fig. 12) or " resting " conidia (Plate V, Fig. 10) or vegetative hyphæ. If kept moist they retain their vitality for over nine months, but if they are completely dried, their germinating power does not last for even a week.

That the " resting "conidia are parthogenetic oospores is very improbable for the reason that they are over twice the size of normal oospores and that from the very beginning of their development they are thick-walled and slightly yellow tinted, while parthogenetic oospores have their origin in thin-walled and hyaline oogonia which, on failing to come in contact with antheridia

after reaching maturity, undergo the same changes, at least outwardly, as they would have if they had been fertilised. The " resting " conidia germinate readily, while the oospores have not been found till now to germinate, except in one doubtful case.

Von Faber[1] in Cacao *Phytophthora* has found oospores but he failed to find antheridia and oogonia. Coleman[2] explains this failure on the ground that the oospores " almost always fill the oogonial cavity so completely that the oogonial wall can be made out only with difficulty." His figures 7, 8, and 9 of Plate XVIII leave doubts in my mind as to their being drawings of true parthogenetic oospores. They seem to be " resting " conidia or chlamydospores, which Rorer has found in diseased fruits. They quite resemble " resting " conidia found in *Ph. parasitica.*

In the *Phytophthora* on *Hevea brasiliensis*, which is considered to be *Ph. Faberi* by Petch and others, oospores have been found but " Oogonienhäute und Antheridien waren nicht vorhanden."[3] From spirit specimens of fruit of *Hevea brasiliensis* attacked by *Nectria* and by *Phytophthora* I have come across globular yellow thick-walled (2μ thick) bodies, in appearance exactly similar to " resting " conidia found in *Ph. parasitica.* They measure 22 to 30μ in diameter, on an average 27μ. These " resting " conidia might have been mistaken as oospores without antheridia and oogonia.

As in *Ph. Phaseoli*[4], the antheridium is the first to be found in the development of the sexual organs. The formation of the antheridium is indicated by the terminal swelling of a hypha, which sometimes prolongs its course to a few microns in length beyond the swelling, or by the swelling of the wall of a

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[1] Von Faber, F. C. Die *Phytophthora*-Fäule der Kakaofrüchte, Arbeiten aus der Kais. Biol. Anst. für Land-und Forstwirtschaft, VII, 1910, p. 201.

[2] Coleman, L. C. *loc. cit.*, p. 74.

[3] Peters, Uber eine Fruchtfäule von *Hevea brasiliensis* in Kamerun. Ber. üb. d. Tätigkeit d. Kais. Biol. Anst. im Jahre 1911. Mitt. Kais. Biol. Anst. 1912, No. 12.

[4] Clinton, G. P., *loc. cit.*, 1907-1908, p. 902.

rather old hypha somewhere in its middle. Thus according to its development the antheridium is terminal or intercalar. The swollen portion of the hypha is soon cut off by one or two septa according as it is terminal or intercalar. The walls of the swelling remain thin or become slightly thicker than those of its stalk. There is no trace of the oogonium till the antheridium is completely developed, or almost so. The oogonium and the antheridium are on the same stalk or on different stalks. When they are on the same stalk the oogonial hypha arises from the base of the antheridium as an ingrowth (Plate VI, Figs. 3 and 4). When the oogonial origin is developed on a separate hypha, it grows towards the antheridial swelling and makes its way into the interior of the latter when it comes in contact with it. In some cases the oogonial hypha swells before penetrating the antheridium (Plate VI, Fig. 14), in rare cases it has been found to indent into the wall of the antheridium before boring its way through it (Plate VI, Fig. 2). Once within the antheridium the hypha continues its growth until the apex is reached. At this stage the swollen apical wall of the oogonial hypha is thinner than its side walls (Plate VI, Figs. 4 to 7), and its apex is also more highly refractive than the rest of the hypha. The swollen head of the oogonial hypha now dissolves the antheridial wall in contact with it and grows out of it. Immediately it emerges it, as a rule, swells out into a sphere or almost a sphere containing oil drops, the oogonium proper. When the oogonium is mature its protoplasm gets differentiated into the oosphere, surrounded by a very thin hyaline wall. Later on changes take place in the oosphere, the thin wall begins to get thickened and contracts a little from the oogonial wall. There is a simultaneous change in the oogonial wall as well ; it also gets thickened and turns yellow. These changes mark the conversion of the oosphere into the oospore, after fertilisation, but the exact moment of fertilisation cannot be ascertained ; at no stage in the development of the antheridium and oogonium has ever been found any special process through which the fertilisation might be effected, probably on account of the way in which the antheridium envelops the oogoni-

um. They are in such intimate contact over a considerable area that nuclear transfer might take place at any time after the penetration of the antheridium by the oogonial origin. After fertilisation, the oogonial stalk within the antheridium is found in the majority of cases to possess a septum or a thick cellulose plug or both ; in those cases in which neither septa nor cellulose plugs have been observed, they might be present just where the antheridium is pierced by the oogonial stalk and so escape observation. The oogonium forms a permanent coating round the oospore which almost fills it ; even after the formation of the oospore the antheridium is not always completely empty of its protoplasmic contents.

I cannot definitely say if the oogonium is ever developed terminally, but in those few cases in which the hyphæ bearing the oogonium could be clearly traced for some distance, it was found that it was on a lateral branch of another hypha (Plate VI, Figs. 10, 15 and 20), or occasionally sessile or what may be termed intercalar (Plate VI, Fig. 11).

Both Jones and Clinton[1] have observed that the oogonial thickening in *Ph. infestans* is influenced by the medium in which it is grown. A similar difference in the oogonial thickenings of the oospores of *Ph. parasitica* is found in French-bean juice agar and oat juice agar. The oogonium in the former medium is slightly thickened, at times very little, smooth and yellow. Within it is seen the oospore, almost filling the oogonial cavity. Very often the enclosed oospore lies in such close contact with some portion of the oogonial walls that it is very difficult to differentiate between their walls at this place. The oogonium measures 15-27μ in diameter, as a rule 18-25μ, and the average of 60 measurements gave a diameter of 23·8μ. The oogonium in oat juice agar is thick-crusted on account of secondary thickening on its wall, orange yellow in colour, and without a sharp contour. At times the thick oogonial wall is so opaque that the enclosed oospore which almost fills the oogonium is only seen on crushing it. These thick-walled dark

[1] Clinton, G. P. *loc. cit.*, 1909-1910, p. 772.

coloured oogonia growing in oat juice agar, look similar to those of *Ph. infestans* found in the same medium by Clinton[1], but they are much smaller. The thick-walled oogonia of *Ph. parasitica* vary from 25 to 35μ in diameter, while those of *Ph. infestans* vary from 34 to 50μ in diameter. Though the oogonia from French-bean juice agar are smaller than those from oat juice agar, this difference in size being due only to the nature of the two media, still the oospores from both these media are of the same size. They vary from 13 to 24μ in diameter as a rule from 15 to 20μ. The average of 60 measurements gave a diameter of 18·6μ. They have a thick wall, about 1 to 2μ in thickness, and are spherical, smooth and hyaline. They have a homogeneous mass of protoplasm, generally containing one or more oil globules.

The antheridia are hyaline, thin-walled and irregular in shape. Those produced in French-bean juice agar are larger than those found in oat juice agar. They are persistent; even in very old cultures they have been found to remain attached to the oospore. The oospore breaks off from its antheridium when roughly manipulated.

The oospores in oat juice agar are embedded in the medium and are, as a rule, confined to those portions containing matted budding hyphæ, which are considered to store reserve material for the fungus. These are invariably empty after the formation of the oospores. Very often the oospore is completely entangled in the network of those hyphæ. The oospores in French-bean juice agar are embedded in the medium, but they are not restricted to any particular portion of it. They are found mixed up with sporangia and "resting" conidia.

The oospores of *Ph. infestans* "are more likely to be found in the upper and drier part of the tube[2]" but the oospores of *Ph. parasitica* have invariably been found in the lower and moister part of the tube and never in the upper and drier. There is as much variability and uncertainty in the production of these oospores as Clinton found in the production of those of *Ph. infestans*. Some

[1] Clinton, G. P. *loc. cit.*, 1909-1910, p. 772.
[2] *Ibid.*, p. 765.

portions of the culture tube contain a lot of them in all stages of development, while in other portions there is no trace of them.

Ph. parasitica oospores have been found in French-bean juice agar and oat juice agar but they have only been obtained when these media were inoculated from cultures growing on a different medium.

Clinton has found it difficult to decide whether the oogonial thread "did not actually penetrate the antheridium and grow through it," but "the optical effect was frequently that of an internal thread." I have not found a single case in which the oogonial origin was not within the antheridium. The microphotographs bear independent evidence to the unique mode of development of the oogonium. One of them (Plate VII, Fig. 1) shows the oogonial thread arising as an ingrowth within the antheridium. Another (Plate VII, Fig. 5) shows a roughly manipulated oospore. At the apex of the antheridium the elliptical opening made by the oogonial thread was distinctly visible. Within the antheridium is seen the passage made by the oogonial thread in the course of its upward progress. Plate VI, Fig. 16, shows an unfertilised oogonium which through rough handling got a little separated from its antheridium. This figure clearly illustrates the exact origin of the oogonium.

It is a matter of common observation that when a hypha penetrates its host cell at right angles to it, under the microscope the penetrating end of the hypha, observed vertically, appears as a continuous chain of rings lying within the cell when the fine adjustment of the microscope is being used. The same phenomenon is observed in the antheridium which is being pierced by an oogonial hypha (Plate VI, Fig. 1). When the oogonium and antheridium are on the same hypha the oogonial stalk broadens out at the place of its origin at the base of the antheridium. All these observations leave no doubt as to the oogonium arising from within the antheridium.

Composition of the Membranes.

According to Mangin[1] the mycelium of the *Peronosporaceæ* can be very easily distinguished from other fungi by the "constant presence of callose, pure or associated with cellulose" which partially or completely plugs the mycelial tubes at intervals. I have failed to find this constant presence of callose in *Ph. parasitica*. No doubt within the hyphæ have been seen refractive, homogeneous, hyaline and generally bi-convex bodies which completely fill the hyphal cavity (Plate V, Figs. 5a, c and d). They arise as ingrowths from the inner walls of the hyphæ (Plate V, Figs. 1, 4d and 5). The hyphal cavity is closed either by one such ingrowth extending from one wall to the other or by two or more arising from the opposite sides and meeting at some point within the hypha. These plugs, unlike the callose plugs found by Mangin[2] in the other *Peronosporaceæ*, have never been observed to originate as annular rings within the hyphæ. They are composed of pure cellulose and not of pure callose or callose associated with cellulose. The presence of callose is indicated by a yellow colour given to it by the iodine reagents for cellulose, *e.g.*, calcium chloride-iodide, iodine and phosphoric acid, iodine and sulphuric acid and Schulze's solution. These reagents have invariably stained the plugs blue or violet without any trace of yellow. Caustic soda and caustic potash easily dissolve callose; ammonia and alkali carbonates swell it and give it a gelatinous appearance; sulphuric acid slowly dissolves it; cupric ammonium hydrate has no action on it even after long treatment with strong acids. These are the reactions given by Mangin[3] for callose. The plugs found in our fungus are not dissolved by caustic soda or caustic potash; they are no way affected by ammonia and alkali carbonates, they are readily dissolved by sulphuric acid; after two hours' treat-

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[1] Mangin, L. Sur la structure des *Péronosporées*. C. R., CXI, 1890, p. 924 and Recherches sur les *Péronosporées*. Bull. Soc. Hist. Nat. d'Autun, VIII, 1895, p. 76.

[2] *Ibid.* Sur la désarticulation des conidies chez les *Péronosporées*. Bull. Soc. Bot. Fr., XXXVIII, 1891, p. 234.

[3] *Ibid.* Sur la callose, nouvelle substance fondamentale existant dans la membrane des cellules des végétaux. C. R., CX, 1890, p. 646.

ment with hydrochloric acid, cupric ammonium hydrate dissolves them completely in a short time. Thus the reactions which helped Mangin to trace the presence of callose, pure or associated with cellulose, in the *Peronosporaceæ* examined by him, have shown us the complete absence of the substance from the plugs found in *Ph. parasitica*. These plugs give all the colour and chemical reactions for cellulose. Mangin has not only found callose plugs in the mycelium of the *Peronosporaceæ*, but also callose depositions on the walls. These depositions as well have been given great importance by him, as being an important distinguishing characteristic of the order. The mycelium of *Ph. parasitica* has been found studded on its inner walls with some deposit which gives all the chemical and colour reactions of cellulose and none of callose. These cellulose deposits are very beautifully seen if the mycelium be first treated with a strong acid, such as phosphoric acid, for a few hours and then treated for a very short time with a weak solution of cupric ammonium hydrate and stained with phosphoric iodide after being well washed with water. The mycelium takes a beautiful blue colour and the studs on it a darker blue, without any trace of yellow in them. This studded mycelium looks quite similar to what is figured by Mangin.[1] This studded deposit of cellulose has also been found in sporangia and "resting" conidia. Mangin has observed no deposit on the sporangia of the *Peronosporaceæ* examined by him, at least he makes no mention of them in his various notes on this subject I have consulted. Callose has been found to be absent even from the septa. The beginning of the formation of the septum is seen as a hazy, ill-defined, hyaline and slightly refractive line, running from one wall to the other in the midst of a mass of protoplasm. When stained with iodine and phosphoric acid the septum stands out clearly by its faint blue colour in the yellow coloured protoplasm. This hazy and ill-defined line, later on becomes sharply defined and stains deep blue with iodine and phosphoric acid. Cellulose thickening then

[1] Mangin, L. Recherches sur les *Péronosporées*. Bull. Soc. d'Hist. Nat. d'Autun, VIII, 1895, fig. 8, p. 88, and fig. 9, p. 90.

begins to take place on the side or sides where the protoplasm is present. If the protoplasm disappears from both the new cells formed by the septum there is no thickening on it. This thickening of the septum is found to be of pure cellulose. It is dissolved by cupric ammonium hydrate and sulphuric acid, no way affected by caustic potash, alkali carbonates and ammonia and stains blue or violet with all the cellulose iodide reagents. This thickening on the septum is found not only in the vegetative hyphæ but also on the septum of the oogonial stalk within the antheridium and on the antheridial septa as well.

These cellulose plugs in hyphæ, and cellulose deposits on their walls and septa, have been observed by me in the hyphæ of *Ph. Faberi* also.

The sporangia are composed of pure cellulose, except their papilla. As already stated they have on their walls irregular cellulose thickenings. At the point of attachment to the sporangiophores generally a thickening has been found, which is of pure cellulose and not of callose as observed by Mangin[1] in the other *Peronosporaceæ*.

The "resting" conidia have been found to possess three walls; the outer, thin and hyaline, is closely applied to the central thick and yellowish wall and escapes observation unless it is stained; the innermost wall is thicker than the outer but thinner than the central wall, its colour is masked by the yellow of the latter, to which it is not closely attached. Very young "resting" conidia apparently stain yellow with the iodide reagents for cellulose, but if they be closely examined a faint coat can be detected around them. In very young intercalar "resting" conidia, which have not become spherical but are ellipsoidal, a quantity of cellulose is collected at the ends. When mature the "resting" conidia colour blue or violet with iodine and phosphoric acid or with any other iodine cellulose reagent, as the outer wall has become thicker. If they be first treated with hydrochloric acid for a couple of hours and then for a short

[1] Mangin, L. Sur la désarticulation des conidies chez les *Péronosporées*. Bull. Soc. Bo . Fr., XXXVIII, 1891, p. 234.

time with cupric ammonium hydrate the outer wall is dissolved. After this treatment iodine and phosphoric acid stains the central thick wall quite yellow and the interior of the conidium appears filled with a blue mass, possibly the dissolved inner wall. If in place of strong cupric ammonium hydrate a weak solution of this reagent is used for a short time, remnants of the outer thin wall are visible and they stain blue with the cellulose colour reagents. Evidently the thicker central yellow wall is of quite a different chemical composition from the other two, which are shown to be of cellulose. Ruthenium red, acidified Bismark brown and alum hæmatoxylin stain the " resting " conidia not very sharply without any previous treatment, on account of the outer coat of cellulose on which these stains have no effect. But when the outer wall is dissolved by cupric ammonium hydrate, acidified Bismark brown stains the central wall brown, which colour is not washed out by hydrochloric acid, while alum hæmatoxylin stains it violet blue very clearly. Mangin gives these stains as specific reagents for compound pectic substances. Ammonia and alkali carbonates have not any action on the thick yellow coloured central wall and therefore it is not composed of pure pectose, but of some compound pectic substance. When treated with sulphuric acid the central wall remains undissolved, even after a long treatment, while the two other walls are completely dissolved in a short time; caustic potash does not immediately dissolve it. These reactions clearly prove the absence of callose from the walls of " resting " conidia.

The composition of the antheridial wall is quite simple. It is constituted of pure cellulose without any callose. It is immediately dissolved by sulphuric acid and cupric ammonium hydrate; ammonia, caustic alkalies and caustic carbonates have no action on it; it readily stains blue with all the cellulose colour reagents.

The walls of the unfertilised oogonium are composed of pure cellulose. After fertilisation the oogonial wall undergoes modification. In the *Peronosporaceæ* investigated by Mangin[1], the

[1] Mangin, L. Recherches sur les *Péronosporées*. Bull. Soc. d'Hist. Nat. d'Autun, VIII, 1895, p. 99.

oogonium at this stage was found to possess two walls; the internal contained only cellulose and no callose, or if at all very little, and the external, on the contrary, contained chiefly callose and little or no cellulose. In *Ph. parasitica* it is also surrounded by two walls but the outer thick wall is not found to be of callose. Congo red,[1] which stains both cellulose and callose, failed to stain the oogonium, though hyphæ, sporangia, "resting" conidia and antheridia were stained. Cold strong sulphuric acid had no action on it, even though the treatment was prolonged for over a week, alkali carbonates and ammonia neither swelled it nor gave it a gelatinous appearance, iodide reagents for cellulose intensified its original yellow colour, it was not stained by nigrosin. The outer thick wall of oogonia of *Sclerospora graminicola* has been found by Mangin[2] to consist of pure callose and under the influence of phosphoric acid this thick wall swells excessively, becomes jelly-like and sets free the oospore, but the oogonial wall of *Ph. parasitica* is not affected by this acid, even after a long treatment. Malachite green, gentian violet, carbofuchsin stain deeply all the parts of the fungus but the stain is washed away by the use of acidulated water from all of them, except from the oogonium. The same selective affinity for the oogonium is shown by Hofmann's violet. Safranin, Kleinenberg's Hæmatoxylin, Delafield's Hæmatoxylin and eosin do not stain the oogonium; iodide reagents for cellulose only intensify its yellow colour. These chemical reactions and stains show that this thick outer wall is composed of a chemical substance other than callose or cellulose.

The possibility of this thick wall being composed of some pectic compound substance was suggested by its staining beautifully red with Ruthenium red, which has been successfully used by Mangin[3] for detecting the presence of compound pectic substances in the

[1] Mangin, L. Sur la callose, nouvelle substance fondamentale existant dans la membrane des cellules des végétaux. C. R., CX, 1890, p. 645.

[2] *Ibid.*, Recherches sur les *Péronosporées*. Bull. Soc. d'Hist. Nat. d'Autun, VIII. 1895, p. 97.

[3] *Ibid.*, Sur l'emploi du rouge de ruthénium en Anatomie végétale. C. R., CXVI, 1893, p. 653.

higher plants. He considers this to be the only reagent for the products of the transformation of compound pectic substances. They are dissolved by the successive action of hydrochloric acid and an alkali. As recommended by Mangin[1] the oospores were at first boiled for half an hour with two per cent. hydrochloric acid and then with two per cent. caustic potash for a long time ; after each boiling they were thoroughly washed. The result was that the outer thick coat of the oogonium was dissolved, exposing the thin inner coat, which had swelled under the treatment. The oospore was lying loosely in it. The sporangia and " resting " conidia had considerably swelled. Iodine and phosphoric acid and the other iodide reagents of cellulose stained blue or violet the swollen internal wall of the oogonium. Eau de javelle completely dissolved the outer thick wall in twenty-four hours. After treatment for an hour or two, it became quite soft and allowed the cellulose stains to reach the internal wall and the oospore. The outer thick coat stained brown with acidified Bismark brown and the stain was not washed away by acids and alcohol ; it also stained violet with alum hæmatoxylin. The former reaction shows that it is neither lignified nor suberised, for the stain given to lignin and suberin by Bismark brown is washed out by acids and alcohol. When treated with seven per cent. caustic potash for a week or so the outer coat is completely dissolved and the inner coat is also completely dissolved or almost so ; in the latter case merely a faint halo is visible round the oospore. This halo becomes just visible by its taking a bluish tinge with phosphoric iodide. This inner wall, therefore, is probably composed of some soluble form of cellulose, similar to that which has been found by M. Hofmeister[2] to stain with zinc-chloride-iodide and to be soluble in five or six per cent. of alkali.

The composition of the outer coat, which is dissolved by seven per cent. caustic potash after a long treatment, by eau de javelle in twenty-four hours and by the successive treatment with hydro-

1 Mangin, L. Nouvelles observations sur la membrane. Bull. Soc. Bot. Fr., XL, 1893, p. 273.
2 *Vide* Mangin, L. *loc. cit.*, p. 278.

chloric acid and caustic potash, which is not dissolved by acids, alkali carbonates or ammonia, which stains red with Ruthenium red, violet with alum hæmatoxylin,[1] and brown with acidified Bismark brown,[2] the stain being not washed out by acids and alcohol, is presumably some compound pectic substance.

The stalk of the oogonium within the antheridium undergoes the same modification as the fertilised oogonium, though not to such a great extent as the latter. This stalk occasionally has a cellulose plug like those found in vegetative hyphæ.

The composition of the walls of the oospore could not be accurately determined. But the outer wall appears to be composed of some compound pectic substance and the inner wall of cellulose; because the solvents of the outer wall of the oogonium dissolved the outer wall of the oospore and the undissolved part of it gave all the reactions of cellulose; because strong sulphuric acid did not dissolve the outer wall of the oospore; again when the oogonium was allowed to be acted upon either by weak solutions of its solvents or by strong solutions for an insufficiently short time the enclosed oospore when treated with the iodide reagents of cellulose stained yellow on the outside and blue in the interior.

During this work I have found chlor-zinc-iodide and iodine and sulphuric acid, commonly used for staining cellulose, to be very unsatisfactory as their action is capricious, but calcium-chloride-iodide and iodine and phosphoric acid recommended by Mangin have given excellent results, especially the latter; with its use the intensity of the stain can be easily regulated by the use of different strengths of iodine solution and the stained slides have been found to keep well for over two months.

Media.

French-bean (*Phaseolus vulgaris*) juice agar and oat juice agar have proved to be the best media. These two media have been

[1] Mangin, L. Sur a constitution de la membrane des végétaux, C. R., CVII, 1888, p. 145.
[2] *Ibid.*, Sur la présence des composés pectiques dans les végétaux, C. R., CIX, 1889, p. 580.

found to be equally successful for cultures of *Ph. Faberi*. Oospores of the castor *Phytophthora* were produced in these media quite luxuriantly, especially in French-bean juice agar. Both these media give a very poor growth of the fungus if they are older than about two months. It has been found advisable to use freshly made cultures. In old media the fungus grows very slowly and produces very scanty aerial mycelium, more scanty sporangia and rarely oospores.

The acidity of French-bean juice agar and of oat juice agar has been found to be +2 Fuller's scale for each. Of course there must have been slight variations in the different sets of these media prepared at different times. The following notes give a brief account of the growth of *Phytophthora* from *Ricinus* on the various media tried.

French-bean (*Phaseolus vulgaris*) *juice agar* (50 + 10 + 500).— Soon after inoculation, the growth was confined to the surface of the medium; in a couple of days a rich aerial growth of the mycelium was produced which eventually filled the whole tube. The hyphæ of the aerial mycelium remained unbranched for long distances; when observed in a mass they presented a regular even surface. The hyphæ on the surface of the medium showed attempts at branching, most of which were abortive, hence an irregular thorny appearance was given to these hyphæ. The submerged mycelium was profusely branched. It also contained irregularly swollen hyphæ. The aerial and submerged mycelium produced a rich crop of sporangia and "resting" conidia. As we have seen oospores were luxuriantly formed, embedded in this medium, but only when it was inoculated with the fungus grown on another medium. This medium was prepared in almost the same way as Lima-bean juice agar by Clinton.[1] Fifty grammes of dried beans were powdered in an iron pestle and mortar, made up with about 300 c.c. of water, then either simmered for half an hour or cooked in the steam of a Koch's sterilizer for half an hour. The latter course was preferable as in the former case there was a likelihood of cracking the vessel if it were of glass or of charring the pounded beans if it were

[1] Clinton, G. P. *loc. cit.*, 1907-1908, p. 898.

a metal one, as the powder did not always get thoroughly wetted by the water used. The liquid was then strained off through a fine wire gauze strainer, and 10 grammes of agar melted in a small amount of water were added to it, the medium was then made up to 500 c.c. by the addition of the requisite amount of water. It was then boiled to thoroughly mix its constituents, strained again through a fine piece of cloth into test tubes and autoclaved.

Oat juice agar (50 + 10 + 500).—Growth at first was not as rapid as on French-bean juice agar. But later on the aerial mycelial growth was as good as in the latter. Both the aerial and submerged mycelium resembled those in French-bean juice agar, but the budding hyphæ of the submerged mycelium were more prominent in this medium, especially near about where oospores were formed. Sporangia and " resting " conidia were freely formed. Oospores were not as luxuriantly produced as in French-bean juice agar. Even in this medium they were found only when it was inoculated with a culture grown on another medium. Stab cultures produced a very poor growth, both of the vegetative and reproductive organs. This medium Clinton found to be successful for the formation of oospores of *Ph. infestans*.[1]

Clinton gives a very elaborate method for preparing this medium but our simplified method has given us a medium in which oospores have been fairly richly formed. Fifty grammes of crushed oats such as are fed to cattle on the farm were cooked with about 300 to 350 c.c. of distilled water for half an hour in the steam produced in a Koch's sterilizer. The cooked material was then strained through a wire gauze strainer; to the liquid was added 10 grammes of agar dissolved in a sufficient quantity of distilled water to bring it up to the required 500 c.c. The medium before being poured into test tubes was boiled again for a short time in order to thoroughly mix the ingredients. The test tubes were then autoclaved.

Maize corn meal agar (50 + 10 + 500).—This medium was prepared by boiling the meal in water and mixing it with agar dissolved in a

[1] Clinton, G. P. *loc. cit.*, 1909-1910, p. 760.

sufficient quantity of water to bring the whole up to 500 c.c. The mixture was brought to the boil and strained through linen before filling the tubes. The aerial growth was copious but at first sterile, sporangia were formed after a week. Submerged hyphæ were irregularly swollen.

Maize corn meal.—This medium was prepared by adding to the meal a sufficient quantity of water to keep the medium moist after it was autoclaved. The fungus growth was very good. A rich crop of sporangia was obtained.

Potato juice agar (150 + 10 + 500).—Growth in this medium was not localised ; it was mostly in the substance of the medium and there was a very sparse aerial growth. The submerged mycelium differed from the aerial in being broader and irregularly branched. Sporangia were formed freely both in the aerial and submerged mycelium but none of these were found to produce zoospores. They germinated conidially without falling off from their conidiophores. They were abnormal both in size and shape and contained a lot of oil globules ; an extreme measurement was 110 to 35μ (Plate V, Fig. 14), but when a bit of the mycelium was transferred to water normal sporangia were produced in twenty-four hours. " Resting " conidia were liberally formed.

Boiled rice.—For the production of aerial mycelium no medium except wheat meal was found to be better. The mycelium did not produce irregularly branched or budding hyphæ. At first only a sterile growth was obtained, but about a month later a few sporangia and many " resting " conidia were produced. When a sterile culture was incubated at about 18°C. a very poor crop of sporangia was produced within a couple of days, possibly on account of the sudden change of temperature. When a bit of the sterile mycelium was transferred to water, sporangia were soon formed.

Wheat meal.—This was prepared in the same way as maize corn meal. It proved superior to boiled rice as it gave a more copious aerial mycelium which readily produced sporangia in abundance.

Bread paste (+10 *Fuller's scale*).—This medium was prepared by mixing powdered stale bread with a sufficient amount of

water to keep it moist after sterilization. Aerial growth was as good as on maize corn meal. A very poor crop of sporangia was produced after a fortnight, but many "resting" conidia were formed. Sporangial growth was as poor in the −5 Fuller's scale bread paste. In neutral and +5 Fuller's scale media, sporangia were produced in a fairly good quantity.

Ricinus leaf juice.—This medium was made by boiling about 15 grammes of green leaves in 100 c.c. of water. The growth was poor, the mycelium had no irregularly budding hyphæ. No sporangia were produced.

Ricinus leaf juice agar (100 + 7 + 500).—The growth was submerged and localised. Vegetative hyphæ branched in a coral-like fashion. Sporangial formation was very poor.

Ricinus seed juice agar (12 + 5 + 500).—This medium was prepared as follows: 12 grammes of seeds, with their hard seed coats removed, were slightly crushed and boiled in about 100 c.c. of water. To the filtered decoction was added 5 grammes of agar, dissolved in water and the whole mixture was made up to 500 c.c. The fungus grew moderately well on this medium. Growth was concentric round the point of inoculation and it was entirely submerged. The vegetative mycelium had only a few swollen or budding hyphæ. Sporangia were copiously produced.

Peptone water (*Dunham's solution*).—There was a moderately diffused mycelial growth. No sporangia were formed.

Peptone Bouillon.—The growth was not so good as in Peptone water; the vegetative mycelium contained abnormally broad and budding hyphæ.

Peptone broth.—The fungus grew very poorly on this medium. Vegetative hyphæ were irregularly branched and budding. Sporangia were formed. The mycelium growing within this medium and on several other media, resembled the sterile, aquatic mycelium of *Pythiacystis citrophthora*, Smith and Smith, grown in dilute prune juice agar (Plate V, Fig. 16).

Groundnut agar.—Twenty seeds of groundnut powdered in a mortar were boiled in 150 c.c. water for two hours. The boiled mixture was

allowed to stand for 24 hours. The supernatant liquid was filtered, mixed with 7·5 grammes of agar dissolved in water and was made up to 500 c.c. The mycelium radiated from the point of inoculation. The growth was fairly good but submerged. The vegetative hyphæ were broad and budding and branched at right angles. They were full of large oil granules. They presented a warty appearance on account of many of the branches failing to make further progress after they had originated. They were at some places so broad that they looked as if they were galled. Sporangia were few and scattered.

Jowar (*Sorghum vulgare*) *agar*.—The medium was made up in the same way as groundnut agar except that 1 ounce of well ground jowar grains were used instead of groundnut seeds. The culture on this medium was in every way the same as on groundnut agar, except that there was a little aerial mycelial growth and that the sporangiferous hyphæ also branched and budded like the vegetative hyphæ but not so much.

Potato slab.—The aerial growth was quite luxuriant but was never found to be fertile. The inoculation succeeded only on the cut surface and not on the peel, just as in the living potatoes.

French-bean powder.—The aerial growth was fairly good. Very few sporangia were produced but there were many "resting" conidia.

Glucose meat-extract agar.—This medium consisted of :—

Extract of Lemco	2 grms.
Sodium chloride	2·5 ,,
Peptone	5 ,,
Glucose	10 ,,
Agar	7·5 ,,
Water	500 c.c.

The growth was wholly within the medium and not localised. The fungus gave the surface of the medium a roughened appearance as if it had bacterial contamination. The hyphæ were fertile, irregularly branched and swollen as in groundnut agar and other media.

Meat-extract gelatine.—The growth was very poor. No sporangia were produced.

Dolichos live beans.—The growth of the mycelium was over one-third inch high. Sporangia and " resting " conidia were formed in abundance.

Prune juice agar.—This medium was prepared by boiling prunes in 100 c.c. water for 5 minutes and adding to the filtered decoction 7·5 grammes of agar dissolved in water, making up the whole mixture to 500 c.c. The growth was not very poor. The mycelium contained irregularly swollen budding hyphæ. Sporangia were poorly formed.

Prune juice agar acidified with citric acid.—On this medium the fungus grew fairly well but the mycelium remained sterile even one month after inoculation. The hyphæ did not contain irregular branches or abormal swellings.

Sterilised slabs of carrot, sweet potato (*Ipomoea batatas*) and of corms of *Colocasia antiquorum* did not give the slightest growth of the fungus.

Cultures were also made on sterilised flies and ants in sterilised water but they gave a very poor growth of hyphæ and sporangia.[1]

Inoculation Experiments with, and affinities of, *Ph. parasitica.*

In order to test the affinity of *Ph. parasitica* with the various species of this genus, a number of inoculation experiments were undertaken on as many of their host plants or their allied species as were available. Inoculations were invariably done by means of suspensions of motile zoospores in distilled water taken from pure cultures. The inoculated plants, seedlings and cuttings were covered with bell jars and the inoculated fruits were placed in moist chambers to keep them constantly in a moist atmosphere. The " checks " were kept in exactly similar atmospheric conditions.

Fruits of Cacao, two species of *Oenothera* and of *Cereus* were obtained through the courtesy of Mr. C. Mahaluxmiwalla, Superin-

[1] *Cf.* Coleman, L. C. *loc. cit.*, p. 71.

tendent of the Victoria Gardens, Bombay ; plants of lilac were kindly supplied by the Superintendent, Kumaun Government Gardens.

Young plants of *Solanum tuberosum* were inoculated with zoospores. The plants showed signs of disease in twenty-four hours. The leaves turned black and shrivelled up as if attacked by *Ph. infestans*, and the plant wilted (Plate VIII A). No sporangia were formed on the diseased parts but if they were transferred to water sporangia were readily produced. When a very tender stem was inoculated, from the diseased area a web of aerial sterile mycelium was produced. The diseased area extended both ways. A thick or an old stem took no inoculation, even when wounded. The disease remained localised on inoculated mature leaves. Tubers could be inoculated only through wounds. They produced a rich aerial growth of mycelium but this was always sterile.

Seedlings of *Solanum lycopersicum*, about four to six inches high, took the inoculation within two days (Plate VIII B). The photo was taken three days after the plant was inoculated. The seedlings soon wilted. No sporangia were produced on them but sections showed the presence of *Phytophthora* hyphæ. When leaves of older plants were inoculated they got blighted and blackened near about the point of inoculation ; the diseased area was always localised, even when growing points of big plants were inoculated. Fruits could not be inoculated, even when wounded.

The inoculation proved fatal to seedlings of *Solanum Melongena*, only when they were about four inches high and bore two or three small leaves. In this case also no sporangia were produced. The effect of the inoculation in the case of big mature leaves was localised.

Lilac plants did not take the inoculation vigorously. Inoculated leaves turned black. Sporangia were produced only when the diseased leaves were placed in water. The leaf buds did not get attacked when they were surrounded by a jacket of water containing motile zoospores. This method was used with success by Klebahn[1] in inoculating leaf buds of lilac with *Ph. Syringæ*.

[1] Klebahn, H. *loc. cit.*, p. 55.

Plants of two species of *Oenothera* took the inoculation quite readily in three days, both on leaves and stems. The inoculated area turned brown at first and then black. Both terminal and intercalar sporangia were produced on the surface.

Salpiglossis variabilis, *Gilia nivalis* and mixed spp., *Clarkia elegans*, *Schizanthus retusa* and mixed spp., and *Fagopyrum esculentum* became victims of the inoculation. Only young seedlings were experimented upon.

Opuntia Dillenii and two species of *Cereus* could not be inoculated even through wounds or on growing points. Cacao fruits and apples, even when wounded, did not take the inoculation. The fungus could not attack leaves and corms of *Colocasia antiquorum*, seedlings of *Lepidium sativum*, cuttings and plants of *Panax*, tobacco, *Cleome* sp. (even when incubated at 18°C.), four species of *Jasminum*, *Phaseolus lunatus* and *Phaseolus vulgaris*. Areca nuts, only when inoculated through wounds, produced a copious aerial mycelial growth in four days (Plate IX B). Sporangia were borne on long stalks, they were not sessile as in *Ph. omnivora*, var. *Arecæ*.[1]

In June, 1912, *Sesamum indicum* was sown in a field where castor was grown the previous year. Stray castor seeds fallen from the pickings of that year had germinated in June. About fifty per cent. of them were attacked by *Phytophthora*. When the seedlings of *Sesamum indicum* grew up amidst these surroundings they were found to be attacked by a *Phytophthora*. The leaves had blackened and got curled exactly like the leaves of potato attacked by *Ph. infestans*. The attack was not very severe, only a few seedlings being killed outright. It remained confined only to seedlings about eight to twelve inches in height. The sporangia resembled in size and shape those of the castor *Phytophthora*. The zoospores successfully inoculated castor seedlings, producing the same effect on them as if they were inoculated with *Ph. parasitica*. Seedlings of *Sesamum indicum* produced the characteristic leaf curl when inoculated

[1] Coleman, L. C. *loc. cit.*, p. 51.

with *Ph. parasitica*. These successful cross inoculations show that the fungus is the same in both these cases.

The infection experiments show that some of the plants that are susceptible to infection by *Ph. omnivora*, *Ph. omnivora*, var. *Arecæ* and *Ph. Faberi* can be inoculated by the *Phytophthora* under investigation as well. Though seedlings of *Clarkia elegans*, *Gilia nivalis* and mixed spp. and *Schizanthus retusa* and mixed spp. died of the inoculation, no sporangia were found on them till they were placed in water for about a day. Sporangia are, however, produced on the surface of these seedlings when they are inoculated by any of the above three named species. Coleman found not only sporangia but oospores as well in the tissues of *Clarkia* inoculated with the areca and cacao *Phytophthoras*, so did de Bary in seedlings inoculated with *Ph. omnivora*. An important difference presented by the castor *Phytophthora* from *Ph. omnivora*,[1] *Ph. omnivora*, var. *Arecæ*,[2] *Ph. Faberi* and from *Ph. Syringæ*[3] as well, lies in the former not inoculating *Cereus* even when wounded; while the latter four readily infect it and produce in its tissues oospores as well. *Lepidium sativum* and *Cleome* are susceptible to the attack of *Ph. omnivora*, but not to that of the castor *Phytophthora*. De Bary failed to inoculate *Solanum tuberosum* and *Lycopersicum* and Coleman succeeded in inoculating only their seedlings and those of *Solanum Melongena*. My results are identical with Coleman's. Even though the castor *Phytophthora* inoculated some of the hosts of *Ph. omnivora*, var. *Arecæ* and *Ph. Faberi*, still no relationship can be claimed for them with the former as it failed to inoculate wounded and unwounded fruits of cacao, and the cacao *Phytophthora* did not cross inoculate seedlings and older plants of *Ricinus*, while areca nuts could be inoculated only through wounds. Sporangia were not sessile as those of the areca *Phytophthora* but they were borne on long stalks.

1 Klebahn, H. *loc. cit.*, p. 69. Himmelbaur, W. Zur Kenntnis der *Phytophthora*, Aus dem Jahrbuch der Hamburgischen Wissenschaftlichen Anstalten, XXVIII, 1910, p. 41.

2 Coleman, L. C. *loc. cit.*, p. 178.

3 Klebahn, H. *loc. cit.* Himmelbaur, W. *loc. cit.*

Whatever affinity the inoculation experiments reveal between the fungus under study and *Ph. omnivora*, *Ph. omnivora*, var. *Arecæ* and *Ph. Faberi*, the study of these fungi in pure cultures shows very little in common between the first and the last three. Hartig[1] found oospores of *Ph. Fagi* in water cultures. His results have been confirmed by de Bary[2] and recently by Klebahn,[3] who studied *Ph. omnivora* from beech seedlings in pure cultures, and by Himmelbaur,[4] whose careful study of *Ph. Fagi*, the cultures of which were supplied to him by Klebahn, and of *Ph. cactorum* have brought him to the conclusion that these two species cannot be merged into each other, under the common name of *Ph. omnivora*, as is done by de Bary. He also got the oospores of *Ph. cactorum*[5] in water cultures. Though I have worked with water cultures of castor *Phytophthora* for months together I have never found the slightest trace of the formation of oospores in these cultures. Klebahn also succeeded in growing *Ph. omnivora* on sterilised potatoes and carrots, on which media not only sporangia were formed but apparently oospores as well. *Ph. Syringæ* has also produced oospores on carrots and Coleman has found oospores of *Ph. Faberi* on sterilised potatoes. The castor *Phytophthora* thus disagrees with these fungi by not growing on sterilised carrots and by producing on sterilised potatoes like *Ph. omnivora*, var. *Arecæ* only sterile mycelium and that too only when inoculations were made on the cut surface or on the wounded rind. *Ph. omnivora*, var. *Arecæ* luxuriantly produced sporangia when grown on sterilised flies and a rich sterile mycelium on boiled rice. The castor *Phytophthora* thus differs from this fungus in growing very poorly on sterilised flies and producing sporangia and "resting" conidia on boiled rice.

The most important difference presented by the castor *Phytophthora* from these and all the other species of this genus, except *Ph.*

[1] Hartig, R. *loc. cit.*, p. 45.

[2] De Bary, A. Untersuchungen über die Peronosporeen und Saprolegnieen. Beiträge zur Morph. und Physiol. der Pilze, 1881, p. 22.

[3] Klebahn, H. *loc. cit.*, p. 71.

[4] Himmelbaur, W. *loc. cit.*, p. 50.

[5] *Ibid.*, p. 54.

Colocasiæ[1], lies in the development of the sexual organs. In no other *Phytophthora* except *Ph. Colocasiæ* has the oogonium been invariably found either to originate from within the antheridium or to arise from a separate stalk which penetrates the antheridium and grows through it. Clinton, indeed, is not certain that in *Ph. Phaseoli*[2] the oogonial hypha may not sometimes penetrate the antheridium. Judging from his microphotographs it seems that the oogonial stalk lies within the antheridium but his earlier drawings of these oospores are different from the microphotographs. Hartig[3] has found in *Ph. Fagi*, oogonia in rare cases seated on antheridia and from his observations of these sexual organs, evidently mature, he supposes the base of an oogonium to have blown out into an antheridium. He says: "Ausnahmsweise und zwar vielleicht dann, wenn in nächster Nähe des intercellularen Oogoniums kein anderweites Mycel sich findet, von dem aus die Entwickelung des Antheridiums erfolgen kann, schwillt der Oognienträger unmittelbar unter dem Oogonium blasig an und wird direct zum Antheridium, wie Fig. 24b zeigt. So deute ich wenigstens die zuweilen auftretenden Stellungen des Oogoniums auf dem Antheridium selbst." The figure to which Hartig refers bears some resemblance to our figures; there seems therefore some probability that the oogonium which he found seated on an antheridium had arisen from within it. De Bary does not make any mention of the type of antheridium observed by Hartig. Klebahn who has recently studied *Ph. omnivora* (obtained from seedlings of beech), in pure cultures, also does not seem to have come across such an antheridium, for he makes no mention of it, nor does Himmelbaur in his more recent researches on *Ph. Fagi* and *Ph. cactorum* refer to it. But supposing what Hartig calls an hypogynous antheridium to be in reality an antheridium, through which the oogonium has originated, still the castor *Phytophthora* has other points of disagreement from this fungus, besides those already found from inoculation experiments and from the study of their growth on artificial cultures. Hartig,

[1] See footnote 6 on p. 189.
[2] Clinton, G. P. *loc. cit.*, 1907-1908 and 1905.
[3] Hartig, R. *loc. cit.*, p. 49.

de Bary, Klebahn and Himmelbaur have found oospores being developed in the ordinary way, *viz.*, by the sexual organs arising either from two different branches or from two different stalks of the same branch and the antheridium meeting the oogonium to fertilise it by means of a fertilising tube, while the formation of a hypogynous antheridium was only occasionally met with by Hartig. On the other hand in our *Phytophthora* this customary mode of development of the sexual organs has never been found. Again the oospores are smaller than those of *Ph. omnivora*. De Bary does not give any measurements of oospores of *Ph. omnivora* but he says that they fill two-thirds or four-fifths of the oogonium, which ranges between 24 and 30μ in diameter. Klebahn gives no measurements at all. Himmelbaur found the oospores of *Ph. Fagi* to be yellowish and ranging between 22-30μ in diameter, and those of *Ph. cactorum* brownish and measuring 30-40μ in diameter. While the oogonia of the castor *Phytophthora* measure as a rule 18-25μ, on an average 23·8μ, if we exclude the secondary thickness formed on the walls of oogonia found in oat juice agar; and the oospores are hyaline and measure 15-20μ in diameter, on an average 18·6μ. The oospores of the castor *Phytophthora* being both lateral and intercalar would seem to offer a point of difference from *Ph. omnivora*, but much importance cannot be given to this; both Hartig[1] and Klebahn[2] have found them to be mostly terminal in *Ph. Fagi* while Himmelbaur[3], who had the pure culture of this fungus from Klebahn, found them to be mostly intercalar.

From Coleman's figures it cannot be definitely determined whether or not the antheridium of *Ph. omnivora*, var. *Arecæ* contains the oogonial stalk within it; from his description of the mode of fertilisation it is quite evident that it takes place by means of a fertilising tube, which has never been found in the *Phytophthora* on castor. Again the oospores of the former are much larger; they measure 26-30μ in diameter.

[1] Hartig, R. *loc. cit.*, p. 49.
[2] Klebahn, H. *loc. cit.*, p. 72.
[3] Himmelbaur, W. *loc. cit.*, p. 50.

Ph. Faberi not only shows points of disagreement in inoculation experiments and in its growth on artificial media but also in its oospores. Both von Faber and Coleman consider them to be parthogenetic, as they failed to find antheridia; but Rorer[1] has found them as his figure (Plate XVIII, Fig. 14), distinctly shows the presence of an antheridium. He has also found chlamydospores, which neither of the former investigators seem to have found. Perhaps as already stated they have mistaken these chlamydospores for parthogenetic oospores. According to von Faber the oospores measure 45μ in diameter, while Coleman says they are 22-45μ in diameter. Rorer measures them between 38 and 40μ and the chlamydospores between 30 and 50μ. Whichever of these measurements are correct, they show that the oospores are much bigger than those of the castor *Phytophthora*.

Apart from the already noted differences that exist between *Ph. Syringæ* and our *Phytophthora* there are also those of sporangia and oospores. The sporangia of *Ph. Syringæ*, unlike those of the other species of the genus, have flat lid-like papillæ instead of raised semiglobular papillæ. The oospores are yellowish and much bigger. According to Himmelbaur they are 30μ in diameter and according to Klebahn 18-30 × 17-25μ.

As we have seen *Ph. Phaseoli* perhaps offers some resemblance in the mode of the development of its oospores to the *Phytophthora* on castor. But a study of these two species leaves no doubt as to their being quite different from each other. The mycelium of the American fungus[2] is only intercellular; the hyphæ emerge singly or several in a cluster solely through stomata; the conidiphores are simple or, unlike its Indian ally, more commonly branched at the base; they measure 300-475μ in length; while those of *Phytophthora* on castor measure, as a rule, 100-300μ. Again the former have on them half a dozen or more nodulose swellings which show the points of attachment of sporangia and the sporangia measure 17-35 × 28-42μ. These swellings have never been found on the coni-

[1] Rorer, J. B. *loc. cit.*

[2] Thaxter, R. A new American *Phytophthora*. Bot. Gaz. XIV, No. 11, 1889, p. 273.

diophores of the latter as they bear only single terminal sporangia which are bigger. The antheridia[1] differ from those of the castor *Phytophthora* in being temporary ; the oogonia are much bigger, as they measure 23-38μ in diameter and loosely envelope the oospores, which measure 26-28μ in diameter and possess a thicker wall. In corn meal juice agar, Clinton[2] found practically no aerial growth but a slight embedded growth in which oospores were developed. But our fungus formed a fairly good aerial growth producing sporangia. Oospores were never developed in this medium. Again in corn-meal there was practically no growth, but on this medium the castor *Phytophthora* gave a copious aerial growth.

Ph. Thalictri, which Clinton[3] suspects to be identical with *Ph. Phaseoli*, differs from our fungus in having larger and branched conidiophores, smaller sporangia and bigger and more thick-walled oospores, which do not differ materially from those of *Ph. Phaseoli*. The oogonia measure 25-33μ, are reddish brown and loosely envelope the oospores, which have 3-4μ thick walls and are 18·5-25μ in diameter.

Ph. infestans presents no point of agreement ; the sporangiophores are branched and nodulose, the sporangia are smaller (27-30 × 15-20μ) and the oogonia and oospores much bigger (34-50μ and 24-35μ respectively).

There is absolutely no relationship between the *Phytophthora* on castor or *Ph. Colocasiæ* and *Ph. Nicotianæ*, as apart from morphological differences, the castor *Phytophthora* failed to inoculate tobacco seedlings and *Colocasia antiquorum* and the parasite of the latter did not cross-inoculate seedlings and plants of castor.

It is clear from all that has been said above that the *Phytophthora* on castor is quite different from the known species of this genus. I therefore propose to name it *Ph. parasitica* nov. spec.

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[1] Clinton, G. P. *loc. cit.*, 1905, p. 293.
[2] *Ibid.*, *loc. cit.*, 1907-1908, p. 905.
[3] *Ibid.*, p. 894.

Phytophthora Parasitica nov. spec.

Maculis amphigenis, orbicularibus, centro arescentibus, dilute umbrinus, anulatis, solitariis vel confluentibus ; mycelio intermatricali, ex hyphis inter-et intracellularibus, primo continuis tandem septatis, 3-9μ crassis, constantibus ; haustoriis sparsis, digitatis vel subglobosis, raro ramosis, sporangiophoris 100 to 300μ longis, sporangis terminalibus, interdum intercalaribus vel lateralibus, plerumque ovoideis, subinde globosis 25-50 × 20-40μ ; zoosporis 8-12 × 5-8μ ; sporis perdurantibus globosis, flavidis, 20-60μ, membrana crassa, levi ; oogoniis et antheridiis in vitro cultis ; oogoniis intercalaribus vel lateralibus, globosis, levibus vel rugosis, melleis, 18-25μ (23·8μ), pedicellis per antheridia penetrantibus ; oosporis globosis, 15-20μ, (18·6μ), membrana crassa, mellea, levi.

Hab. in foliis seminisque Ricini communis et Sesami indici. Indiæ orient.

Note.—While this paper was in the press *Sida cordifolia* from Dacca and *Sesamum indicum* from Saharanpur were reported to be attacked with *Phytophthora*.

In nature the parasite on *Sida cordifolia* has been found only on the flowers. Healthy flowers, leaves and stems of this host were inoculated with *Ph. parasitica*. The inoculation proved successful only in the case of flowers. It seems, therefore, not improbable that the species concerned in the Dacca specimens was *Ph. parasitica*.

The diseased leaves of *Sesamum indicum* have characteristic round spots which are either solitary or confluent. On the upper surface they have a brownish centre, surrounded by a whitish pale or dull green area which in turn has a brown areola. On the lower surface the brown centre is surrounded by a more dull green area which has an areola of a pale brown colour. Sporangia borne on long sporangiophores, which resemble those of *Ph. parasitica* on castor leaves, are found on the upper surface. They are slightly smaller than those found on castor leaves but they agree well in shape.

EXPLANATION OF THE PLATES.

PLATE I.

Upper and lower surfaces of a diseased castor leaf.

PLATE II.

Fig. 1. Surface view of a castor leaf showing sporangiophores emerging through stomata and between the cell-walls of contiguous epidermal cells.—Somewhat diagrammatic. Drawn by Dr. E. J. Butler. X 320.

„ 2. Transverse section of the stem of a castor seedling showing the presence of hyphæ in the vessels and in the bundle sheath. Drawn by Dr. E. J. Butler. X 320.

„ 3. Longitudinal section of a pitted vessel showing a hypha leaving through a pit. X 640.

„ 4. Transverse section of the woody portion of the pericarp of a fruit showing the hyphæ boring through the lignified cell-walls by means of fine projection tubes. X 640.

„ 5. Transverse section made at the collar of a castor seedling showing palmately branched hyphæ in cells of the cortex. X 640.

„ 6. Transverse section of a stem of a castor seedling showing hyphæ swelling into globular heads just underneath the epidermis before breaking through it. X 405.

„ 7. Transverse section of a stem of a castor seedling. A cluster of hyphæ breaking through the epidermis. On the left is the swollen head of a hypha just below the epidermis. X 405.

PLATE III.

Fig. 1. A sporangium producing a highly branched vegetative mycelium when kept in the dark for twenty-four hours. (From a water culture.) X 100.

„ 2. Transverse section of a stem of a castor seedling. A is an intracellular branch of an intercellular hypha. At B are finger-shaped haustoria. X 405.

„ 3. Button shaped haustoria. X 405.

„ 4. A branched haustorium. X 405.

„ 5. Transverse section of a castor leaf. Two germ-tubes entering it through a stoma. X 405.

Fig. 6. A germ-tube entering a castor leaf by breaking through the upper wall of its epidermal cell. X 405.

„ 7. A germ-tube entering a castor leaf between the walls of two contiguous epidermal cells. X 405.

Figs. 8—11. Surface views of castor leaves showing the three ways in which the germ-tube enters the leaf. In Fig. 10 the germ-tube crosses over a stoma before entering the leaf. X 405.

PLATE IV.

Fig. 1. Papilla swelling into a vesicle in which the sporangium is discharging mature zoospores. (Fixed with iodine.) X 405.

„ 2. An advanced stage of the same. (Fixed with iodine.) X 405.

„ 3. The vesicle has dissolved; the zoospores are still clumped together. (Fixed with iodine.) X 405.

„ 4. The papilla has dissolved but the zoospores are still within the sporangium. X 405.

„ 5. The discharge of zoospores in the normal way. The zoospore that is just out of the sporangium is linked to the one immediately behind it by a protoplasmic neck. X 640.

„ 6. Zoospores. A shows two zoospores linked together by a protoplasmic neck and possessing two cilia. X 640.

„ 7. A zoospore which came to rest at 10-20. X 640.

Figs. 8—24. Successive stages in its germination. Fig. 8 drawn at 10-35. Figs. 9 to 14 drawn at intervals of five minutes. Figs. 15 to 24 drawn at 11-40, 11-45, 12, 12-35, 12-45, 1-20, 1-40, 2, 2-25 and 3-15 respectively. X 640.

Fig. 25. Germination of zoospores by two germ-tubes. X 640.

„ 26. A motile mass of protoplasm with five visible cilia. (Freehand drawing.)

„ 27. The same in its resting stage. Drawn at 10-30. X 405.

Figs. 28—34. Successive stages of its germination. Observe the gradual disappearance of the outer wall. These figures were drawn at 10-45. 10-55, 11-5, 11-25, 12-10, 1 and 3 respectively. X 405.

Fig. 35. A sporangium germinating into a secondary sporangium. X 405.

„ 36. A bi-papillate sporangium. X 405.

„ 37. A sporangium producing in water cultures secondary and tertiary sporangia when kept under normal conditions for twenty-four hours. X 100.

PLATE V.

Fig. 1. Typical aerial mycelium growing on French-bean juice agar and oat juice agar. (*a*) A "resting" conidium, (*b*) an intercalar sporangium. At (x) are cellulose deposits on the inner walls of the hypha. They have partially closed the hypha. The lowermost sporangium also shows a cellulose deposit on its inner wall and just above its point of attachment to the sporangiophore. X 405.

Fig. 2. Hyphæ on the surface of the same two media.
,, 3. (a) Submerged hyphæ in the same two media. X 405. (b) Typical swollen budding hyphæ near about oospores in oat juice agar. X 405.
,, 4. Different kinds of septa. In (d) two cellulose plugs (x) grow in from the opposite walls which would have ultimately closed the hyphæ at this point, (e) is a bit of an old hyphæ growing in the substance of a medium showing septa at the origins of two branches. X 640.
,, 5. Cellulose plugs in hyphæ. In (a) x_1 completely closes the bore of the hyphæ. At x are three plugs which almost close it. In (b) x is an ingrowth which increases in size by caplike deposition of cellulose on it. In (c) are two ingrowths arising from the opposite walls and meeting each other thereby completely closing the hypha. In (d) x_1 is a thick cellulose plug. At x is a cellulose ingrowth of the inner wall of the hypha. X 640.
,, 6. Branches arising through septa. X 640.
,, 7. "Resting" conidium. X 405.
,, 8. "Resting" conidium germinating. X 405.
,, 9. "Resting" conidium. X 405.
,, 10. "Resting" conidium giving rise to secondary "resting" conidia. X 100.
,, 11. Immature intercalar "resting" conidium. X 405.
,, 12. "Resting" conidium germinating and producing a sporangium.
,, 13. Intercalar "resting" conidium. X 405.
,, 14. Abnormal sporangium germinating conidially; from potato juice agar. X 405.
,, 15. Germinating internal sporangium in the pericarp of a fruit. X 405.
,, 16. Portion of the mycelium from Peptone Broth. X 405.

PLATE VI.

Fig. 1. Antheridium which is just being pierced by an oogonial hypha underneath it; observed vertically downwards. X 625.
,, 2. Oogonial hypha indenting an antheridium. (Freehand drawing).
Figs. 3 and 4. Oogonial hypha originating within the antheridium. X 625.
Fig. 5. Oogonial hypha that is lying within the antheridium after penetrating it. X 625.
,, 6. Same as above, the antheridium is intercalar. Optical section. X 625.
,, 7. Same as Fig. 5. X 625.
Figs. 8—11 More advanced stages of Figs. 5-7. The oogonial hypha boring its way out of the antheridium. Figs. 8 and 9. X 625.
Fig. 10. The oogonial hypha is on a lateral stalk. X 625.
,, 11. A mature intercalar oogonium. The septa in the antheridium have cellulose thickenings on them. X 1165.
Figs. 12—16 and 17—22. Mature oospores. X 1165.

Fig. 12. Ooognium originating within the intercalar antheridium. Optical section.

,, 13. Oogonium borne on a short stalk, not sessile on the antheridium.

,, 14. Hypha bearing the oogonial stalk within the antheridium swelling at its point of entrance.

,, 15. Oogonium borne on a lateral branch, the point of entrance of the oogonial hyphæ is clearly visible. The hypha enters the antheridium from below and leaves it at its apex. The dotted lines indicate the hypha underneath the antheridium.

,, 16. Oogonium separated from antheridium which persists enclosing its stalk.

,, 17. Optical section of an oogonium originating from the base of an antheridium.

,, 18. Optical section of an oogonium developed from a separate oogonial stalk piercing the antheridium.

Figs. 19 and 21. Optical sections, same as Fig. 17. In Fig. 19, there is a cellulose plug in the oogonial stalk.

Fig. 20. Hypha bearing oogonial stalk penetrating the antheridium from above. The dotted lines show the stalk within the antheridium.

,, 22. Same as Fig. 15.

PLATE VII.

Fig. 1. Oogonial thread (O) originating within the antheridium (A). X 500.

,, 2. Mature oospore from French-bean juice agar. Oogonium arising within the antheridium. X 400.

,, 3. Mature oospore from French-bean juice agar. Hypha bearing the oogonial stalk penetrating the antheridium on the left. X 400.

,, 4. A group of mature oospores from French-bean juice agar. X 400.

,, 5. A mature oospore from French-bean juice agar roughly manipulated. The passage made by the oogonial stalk within the antheridium is clearly visible. X 400.

,, 6. Oospore from oat juice agar. X 400.

,, 7. O. Oospores from French-bean juice agar.
R. "Resting" conidia. X 400.

,, 8. "Resting" conidia. X 400.

PLATE VIII.

Fig. A. Seedling of Potato. The central shoot marked with an arrowhead was inoculated. The other two shoots served as controls. Photo taken five days after inoculation.

,, B. Seedlings of tomato. On the left is the inoculated seedling; the other was kept uninoculated as a check. Photo taken three days after inoculation.

PLATE IX.

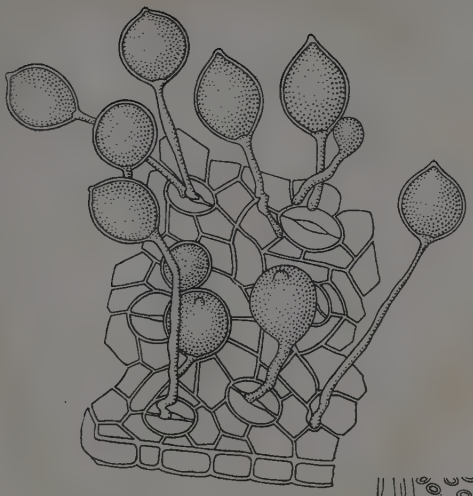
Fig. A. A pot containing a tobacco seedling on the left and a castor seedling on the right. Both were inoculated at the same time. Photo taken two days after inoculation.

,, B. An areca nut inoculated through a wound. Photo taken six days after inoculation.

PLATE X.

A castor seedling inoculated by surrounding it in a jacket of water containing motile zoospores. Photo taken three days after inoculation.

RICINUS LEAVES ATTACKED BY PHYTOPHTHORA PARASITICA.



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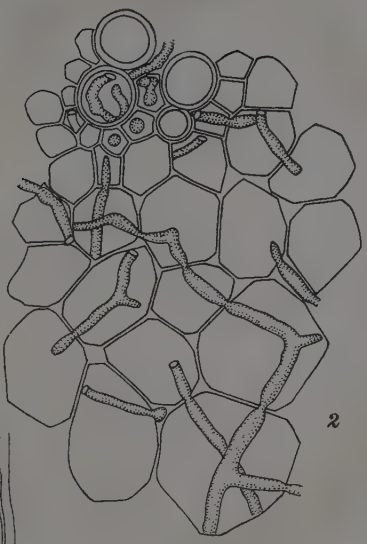

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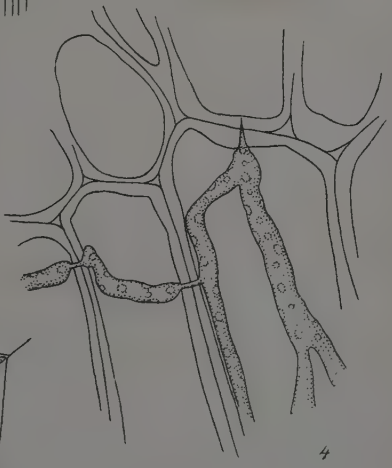

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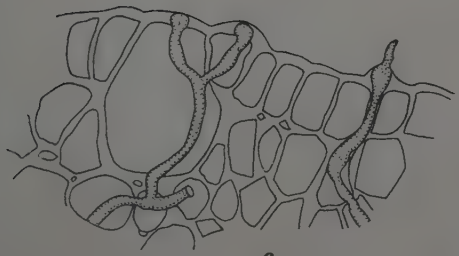

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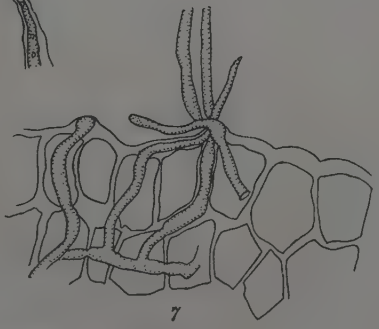

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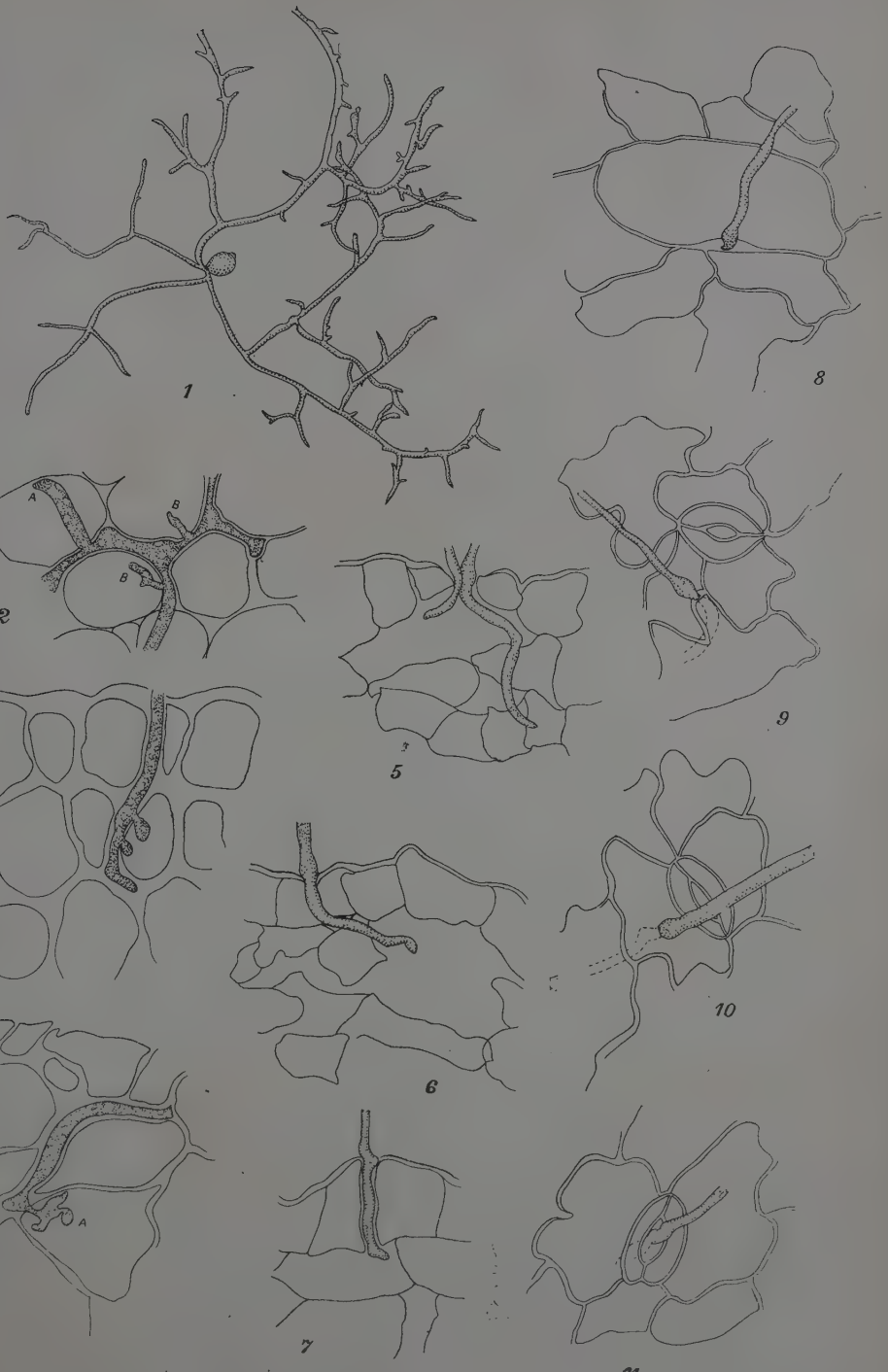
Phytophthora parasitica.

PLATE III.



K. D. Das.

Phytophthora meadii

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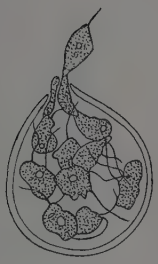
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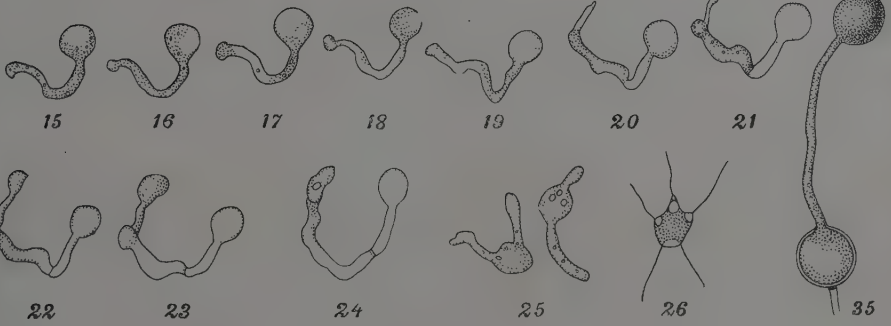
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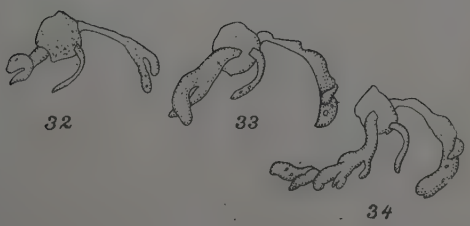
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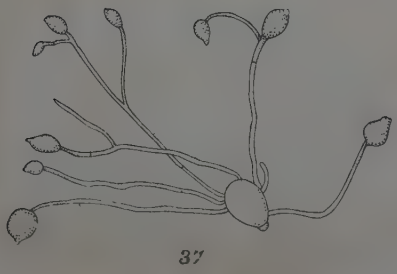
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PLATE V.

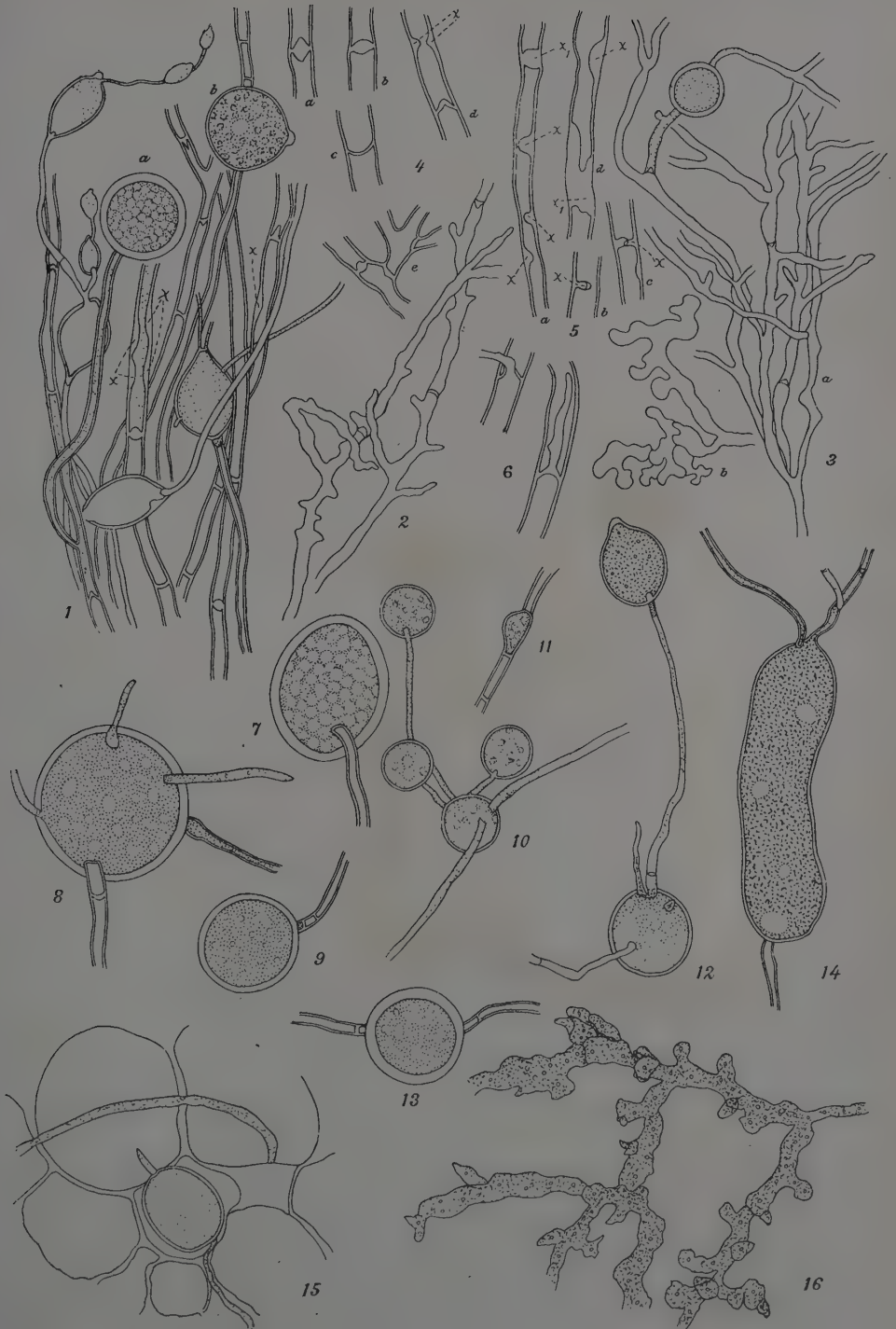
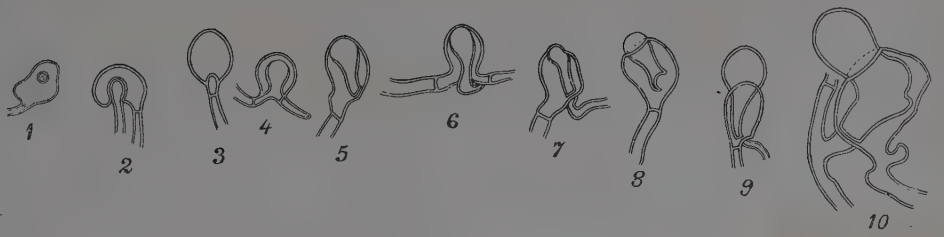
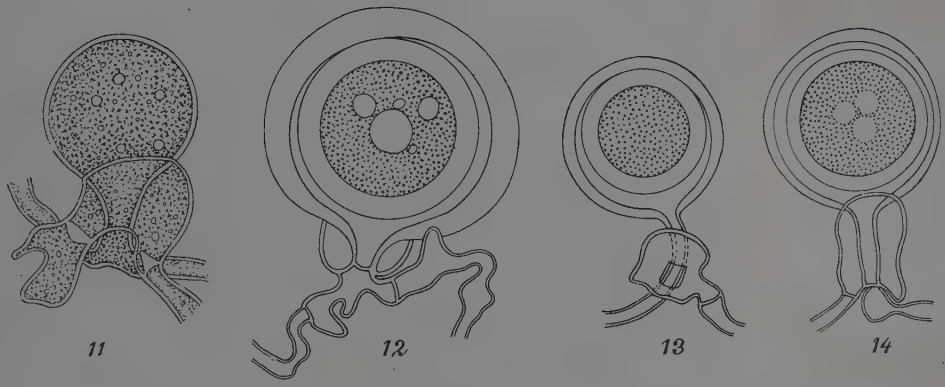
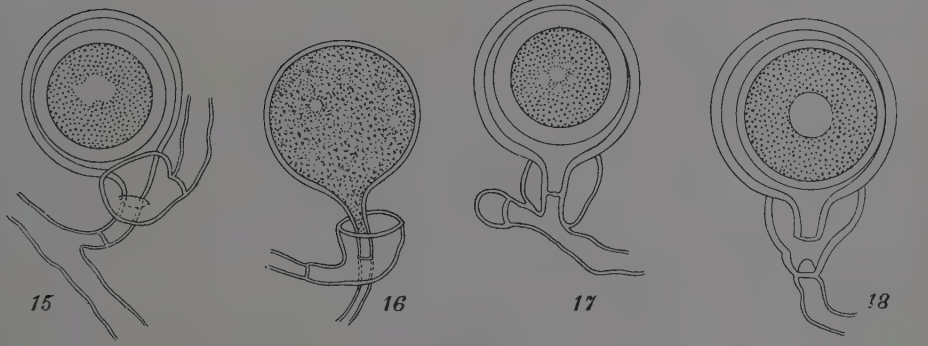
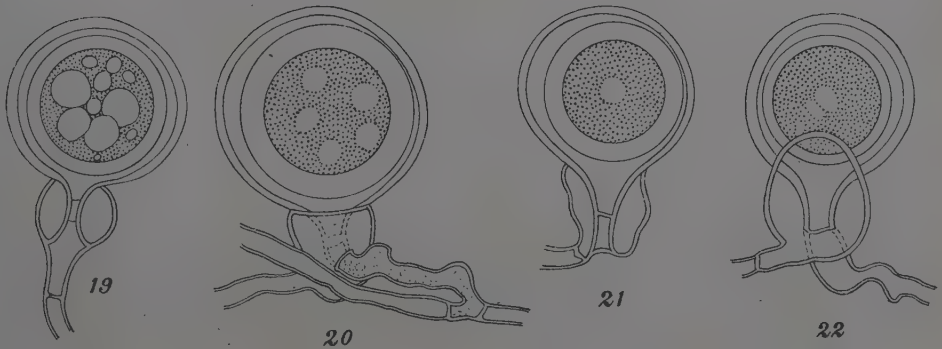


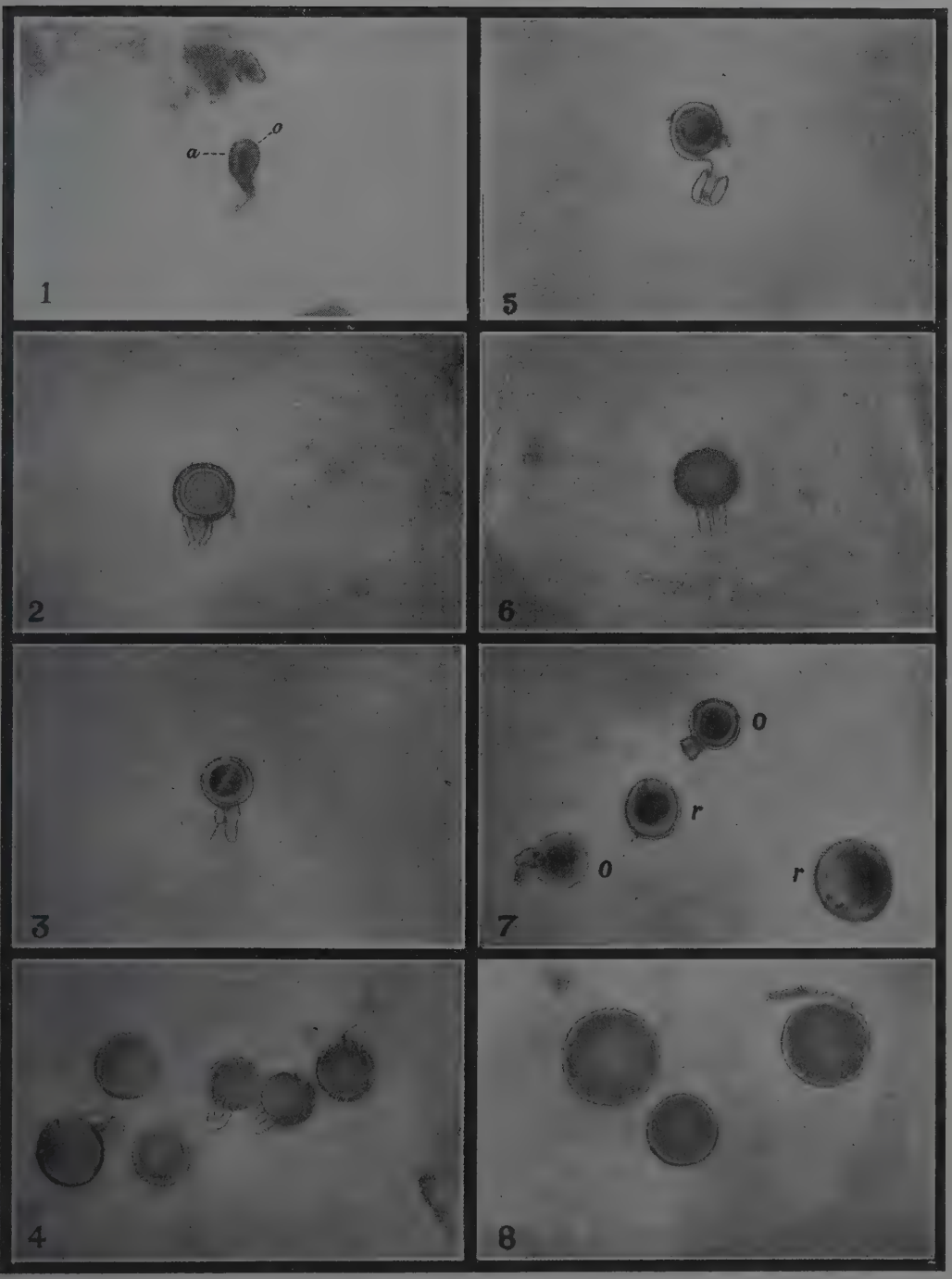
PLATE VI.



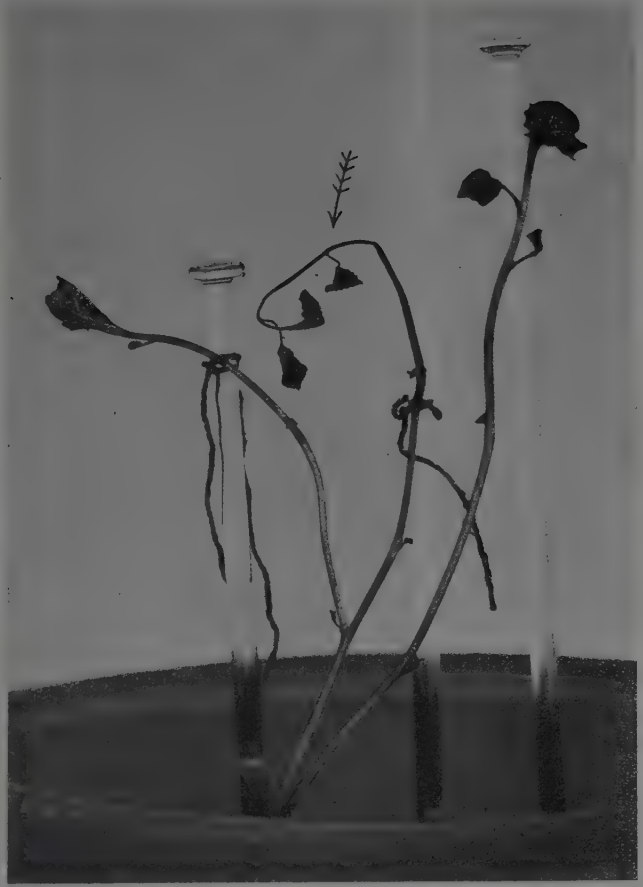








Phytophthora parasitica.



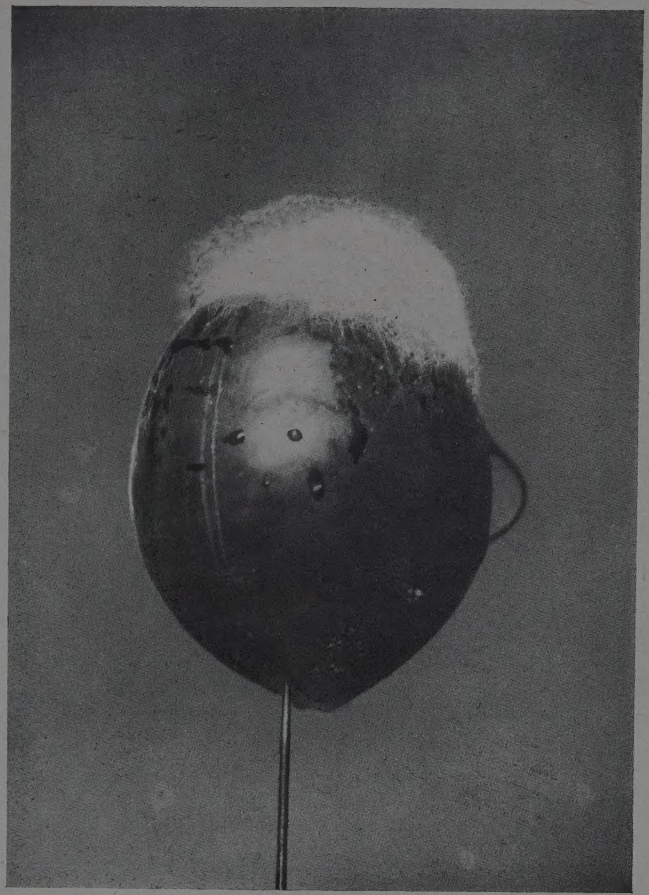
A

B

Phytophthora parasitica.
Inoculations on potato (A) and tomato (B).

PLATE IX.

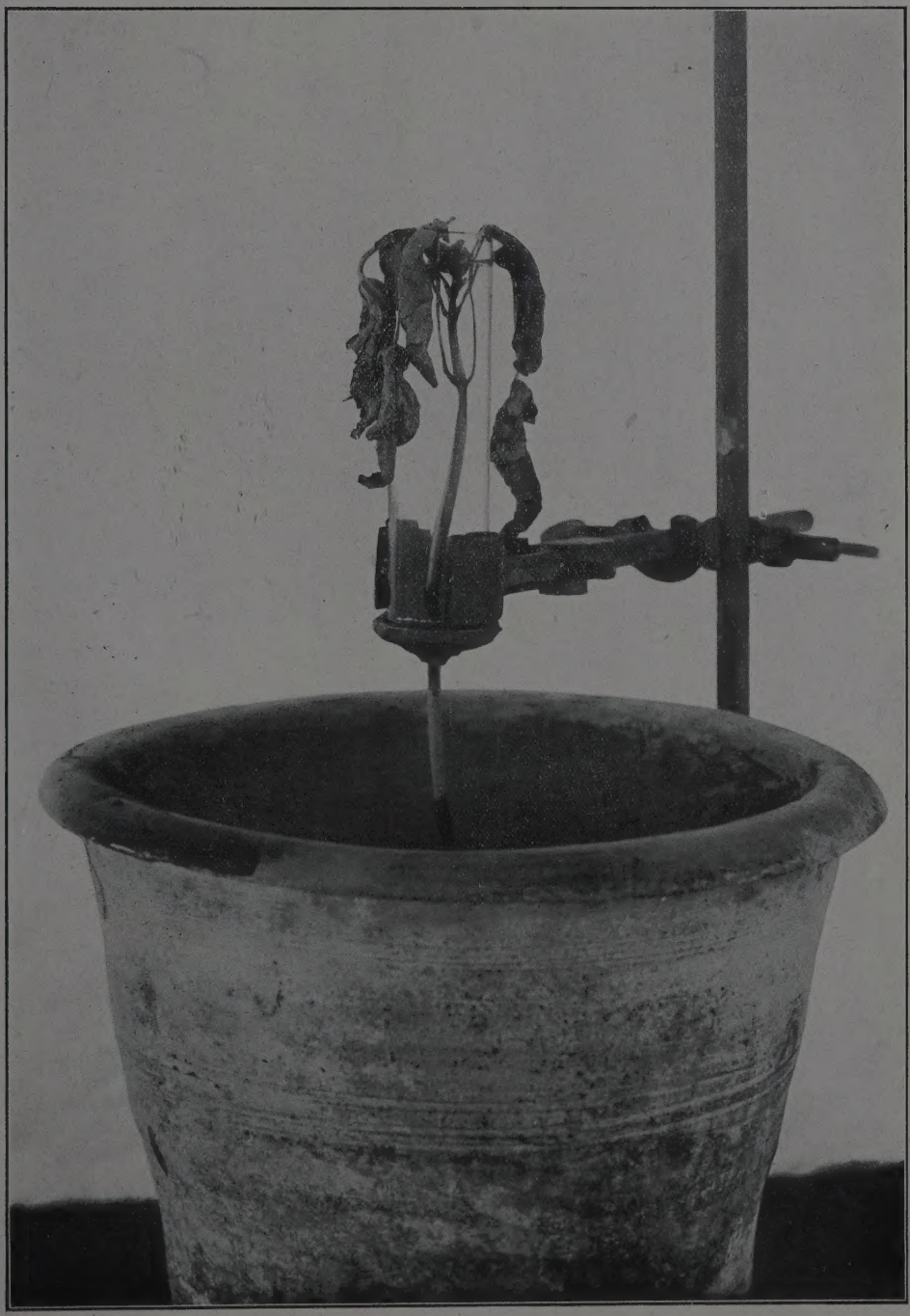
A



B

Phytophthora parasitica.

Inoculations on tobacco and castor (A) and arecanut (B).



Castor seedling inoculated with *Phytophthora parasitica.*